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Evaluation of Haematinic Potential of a Herbomineral Formulation (HMF-TE) in Haloperidol Induced Anaemic Rats

Ashish Trivedi, S.H. Mishra*

Pharmacy Department, Faculty of Tech and Engg., The M.S. University of Baroda, kalabhavan, Baroda, Gujarat, India- 390002.

***Corresponding author's E-mail:** shmishra48@rediffmail.com*

ABSTRACT

Iron deficiency anemia is one of the most common nutritional disorders worldwide, especially in India where 87% of pregnant women suffer from anemia and out of these about 10% have severe anemia (H < 80 g/l). In the present study a new modified herbomineral formulation (HMF-TE) containing two natural mineral sources was prepared in laboratory along with different supportive herbs. This formulation was evaluated for its haematinic potential in haloperidol induced anemic rats. The formulation (HMF-TE) exhibited significant haematinic potential by increasing parameters like haematocrit value, hemoglobin concentration, RBC count, MCV, MCH and MCHC.

Keywords: Iron deficiency anaemia, haloperidol, herbomineral, haematocrit

INTRODUCTION

Anemia is the most common pathological disorder affecting a large group of population of developing countries with varied prevalence, etiology and degree of severity. In a report of World Health Organization, iron deficiency is stated as the most common type of anemia estimated to affect approximately 2 billion people worldwide (1). The world continues to lose a mother a minute, largely from preventable causes (2). Iron deficiency anemia is one of the most common nutritional disorders worldwide, especially in India and other developing countries. Young children and women in the reproductive age group are the most vulnerable to iron deficiency anemia. Surveys in different parts of India reveal that 87% of pregnant women suffer from anemia and about 10% have severe anemia (H < 80 g/l). Variations in the prevalence rates of anemia are seen within the country with the lowest prevalence of 33% being reported from Andhra Pradesh to the highest of 98% in Rajasthan (3).

The treatment of anemia depends on the confirmed diagnosis and severity of the disease. It includes iron therapy (oral or parenteral), iron polymaltose complex, folic acid and vitamin B12 supplement, erythropoietin, bone marrow transplantation etc. The most prominent complication of oral iron therapy is gastrointestinal distress, for these patients abdominal pain, nausea, vomiting, or constipation often leads to non-compliance (4). Chronic oral administration of additional iron failed to correct the anaemia and instead resulted in accumulation of iron in duodenal enterocytes. This has brought to focus the issue of exposing the intestine to large amounts of supplementary iron which may generate free radicals via the Fenton reaction leading to per-oxidative damage of the tissue (5). Presumably, copper deficiency was blocking the efflux of iron out of the intestinal cells. The proposed mechanisms implicated ceruloplasmin, a

copper-dependent protein with ferroxidase activity, which is important for iron efflux and the levels are reduced in iron deficiency anemia (6).

As an alternative therapy, Herbal medicines (herbomineral formulations) are also available for anemic condition, mainly for iron deficient anemia. Some formulations like Amyron, Apimore, Geriforte, Stir, Hbcaps, Haem tabs, Efiplus caps, Herboiron caps, Navayasa tabs, Abhraloha tab, Gestone, Neobliss caps etc. are available in market for the purpose. (7).

'Rasoushadhis' (herbomineral formulations) are used in practice by ayurvedic physicians since long with a rare mention of toxicity. It is observed that herb-mineral complexes are more stable and more interactive as compared to plain herb as these lead to faster therapeutic action and have a longer shelf life(8). In the present study a modified herbomineral formulation was prepared in laboratory based on a traditional ayurvedic formulation, which is marketed as Efiplus caps® containing *Shuddha kasis* (a mineral component- ferrous sulphate), powdered herbs of *Cyprus rotundus*, *Piper longum*, *Zingiber officinale* and aqueous extract of *Glycyrrhiza glabra*. The herbomineral formulation, HMF-TE prepared in laboratory was modified by adding *Tamra Bhasma*(a mineral component rich in copper), as a new component to the original combination of Efiplus caps® . *Shuddha kasis* (a mineral component- ferrous sulphate) serves as chief source for iron while as bioavailability enhancer powdered herbs of Cyprus *rotundus*, *Piper longum*, *Zingiber officinale* (9-11) are recommended. The aqueous extract of *Glycyrrhiza glabra* is added to reduce nausea-vomiting and as antioxidant (12).

MATERIAL AND METHODS

Preparation of modified herbomineral formulation *(HMF-TE)*

HMF-TE was prepared in the laboratory by adopting standard procedure stated in the ayurvedic formulary. The components were first procured from standard suppliers and were then evaluated for their purity using standard procedures (13). The *Suddha Kasis* was prepared by the procedure described in *Rasamrta* (14). The mixing of the mineral and plant components were performed in geometrical proportions according to the formula given in table 1. Both the formulations were evaluated phytochemically and also for their haematinic potential in haloperidol induced anemic rats.

HPTLC fingerprint of formulation

TLC studies of HMF-TE showed the presence of alkaloids; glycosides, saponins, sterols etc. HPTLC fingerprint of methanol extract both the formulations was developed as standardization measure for formulation.

Evaluation of haematinic potential of herbomineral formulation Animals and diet

Adult Wistar rats of either sex, weighing range 190 to 225 gms were randomly assigned to 5 groups (A, B, C, D, E) (n =5). Iron deficient diet was prepared in the laboratory as per AIG- 93 of Dytes Co. that contained 35ppm of iron, being very less quantity in feed. The chemical composition of diet includes 640 gm potato starch, 210 gm casein, 80 mL groundnut oil, 30 gm mineral mix and 40 gm vitamin mix (15). Animals were fed above diet approximately 10-20 gm/rat/day during the study and deionized water was given *ad libitum*. The experiment was in accordance to CPCSEA guideline and approved by IAEC.

Anemia induction

Haloperidol injection (2.5 mg/kg/day i.m.) was administered for five days to all the groups to reduce the haemoglobin levels in animals (16). They were kept on iron deficient diet for the whole study. The rats were considered anemic, when their haemoglobin levels fell to 12g/dL.

The following parameters were selected to assess haematinic potential of herbomineral formulations (17) Haemoglobin concentration, Haematocrit value, RBCs (Red blood cells) count, MCV (Mean corpuscle volume), MCH (Mean Cell Haemoglobin) .

Statistical analysis

All the data were analyzed using one way ANOVA and tabulated as mean values of 5 determinants with ±SEM. Nonparametric statistical methods were used to evaluate significance with the probability level at which the null hypothesis was rejected set at P>0.05 (Graph pad prism V.5.0). All the estimations were carried out using standard procedures.

RESULTS

HMF-TE and HMF-TE double dose were evaluated in haloperidol induced anaemic rats. Haemoglobin levels were reduced to 11 g/dL in all the groups on day 5 of treatment (haloperidol i.m.). The drug was given orally for 15 days to check the haematinic potential of herbomineral formulation. Haemoglobin levels were significantly increased (p<0.01 as compared to control) on day 15 of drug treatment. Haematocrit levels were also significantly increased (p<0.01 as compared to

control) on day 15 of drug treatment. MCV was reduced on day 5 and increased significantly ($p<0.05$ as compared to control) on day 20 of study for HMF-TE double dose group. RBC morphology shows irregular cell walled and hypochromic cell on treatment with haloperidol on 5th day as compared to control and this was corrected with the treatment of herbomineral formulation on day 20 of the study.

DISCUSSION

The neuroleptic therapy is associated with anemia and it was reported to decrease in blood level of iron with haloperidol treatment (18), which was further confirmed with the presence of iron deficient anemia by biochemical parameters. The rats treated with haloperidol injection showed anisocytosis and hypochromic cells (19). The peripheral blood smear has been reported to reveal characteristic changes in the size and the hemoglobin content of the red cells, observed in some types of anemia although the degree of anisocytosis is correlated with the severity of IDA (20). Study shows that young rats fed with a low iron diet develop severe hypochromic anemia within 4–5 wk (21).

A decline in the hematocrit and hemoglobin values was reported by Olivetti and co-workers in rats under iron deficient diet control conditions (22).

Chronic administration of iron in anemic rats resulted in accumulation of iron in intestine. Higher mucosal ferritin and lower serum ceruloplasmin ferroxidase activity impaired the mobilization of intestinal iron. This contributed to greater peroxidative stress in the intestine of iron supplemented rats (23). Many proteins play significant role in absorption of iron from intestine (such as hespidin, DMT-1, ceruloplasmin) and also required for efflux of iron from enterocytes (24). Iron supplements require bioavailability enhancer to minimize the side effects. Herbomineral formulations can be used to reduce various side effects as the processing of various herbal juices with already processed and micro fined minerals lead to the formation of herbomineral complexes. This complexes upon interaction with digestive juices, adopt a colloidal form, to get absorbed fast. Sometimes they play a catalytic role facilitating absorption of other nutrients and correcting a disease process (25).

Table 1. Contents of selected (Efiplus caps®) and laboratory (HMF-TE) herbomineral formulation.

Contents	Form of content	Efiplus caps®	HMF-TE
Shuddha kasis	mineral	200 mg	200 mg
Tamra Bhasma	mineral	-	30 mg
Cyprus rotundus	rhizome powder	100 mg	100 mg
Embelica officinalis	fruit powder	100 mg	100 mg
Zingiber officinale	rhizome powder	100 mg	100 mg
Piper longum	fruit powder	100 mg	100 mg
Glycyrrhiza glabra	(water extract equivalent to)	100 mg	100 mg

Table 2. Blood parameters on day 1 of the study.

Group/Parameters	Haemoglobin	Haematocrit	RBCss	MCV	MCH
Control (vehicle)	17.81±1.283	52.89±3.068	$6.91x10^6$±2.89	7.89 $x10^5$±5.66	$2.63x10^5$±1.94
FeFol®	17.06±1.208	51.31±2.675	$6.75x10^6$±4.47	7.65 $x10^5$±5.83	$2.57x10^5$±1.92
HMF-TE	16.90±1.825	50.29±3.752	$6.83x10^6$±6.25	7.42 $x10^5$±3.24	$2.53x10^5$±1.12
HMF-TED	16.75±3.210	49.97±3.677	$6.81x10^6$±8.78	7.32 $x10^5$±6.66	$2.51x10^5$±2.41
Efiplus Caps®	17.25±2.423	47.24±3.237	$6.87x10^6$±7.34	7.53 $x10^5$±2.94	$2.71x10^5$±1.92

All the values are mean of 5 estimations with ±SEM.

*Key: significance level indicated as * $p<0.01$ as compared to control group.*

Table 3. Blood parameters on day 5 of the study.

Group/Parameters	Haemoglobin	Haematocrit	RBCss	MCV	MCH
Control (vehicle)	10.82±1.58	32.04±1.643	$4.47x10^6$±3.52	$7.20x10^5$± 8.24	$2.42x10^5$±2.77
FeFol®	10.22±1.93	30.56±2.838	$4.15x10^6$±1.89	$7.31x10^5$±6.77	$2.44x10^5$±2.23
HMF-TE	10.26±0.87	29.38±3.217	$4.13x10^6$±1.79	$7.47x10^5$±5.39	$2.41x10^5$±2.60
HMF-TED	11.11±2.803	32.46±1.609	$4.45x10^6$±4.61	$7.33x10^5$±6.68	$2.38x10^5$±2.90
Efiplus Caps®	11.16±1.91	31.06±3.120	$4.31x10^6$±1.09	$7.28x10^5$±6.80	$2.36x10^5$±2.70

All the values are mean of 5 estimations with ±SEM.

*Key: significance level indicated as * $p<0.01$ as compared to control group.*

Table 4. Blood parameters on day 20 of the study.

Group/Parameters	Haemoglobin	Haematocrit	RBCss	MCV	MCH
Control (vehicle)	9.27±1.60	27.49±1.906	$4.14x10^6$±2.68	$6.58x10^5$± 5.86	$2.24x10^5$±2.04
FeFol®	12.96±1.29*	38.62±1.216*	$5.13x10^6$±2.24	$7.30x10^5$±2.68	$2.54x10^5$±1.31
HMF-TE	13.38±1.64*	39.23±2.198*	$5.19x10^6$±1.39	$7.67x10^5$±3.90	$2.61x10^5$±1.51
HMF-TED	14.06±1.516*	41.24±2.440*	$5.32x10^6$±3.83*	$8.02x10^5$±7.92*	$2.74x10^5$±2.54*
Efiplus Caps®	12.33±2.18*	38.83±2.027*	$5.13x10^6$±1.76	$7.76x10^5$±3.08	$2.52x10^5$±1.49

All the values are mean of 5 estimations with ±SEM.

*Key: significance level indicated as * $p<0.01$ as compared to control group.*

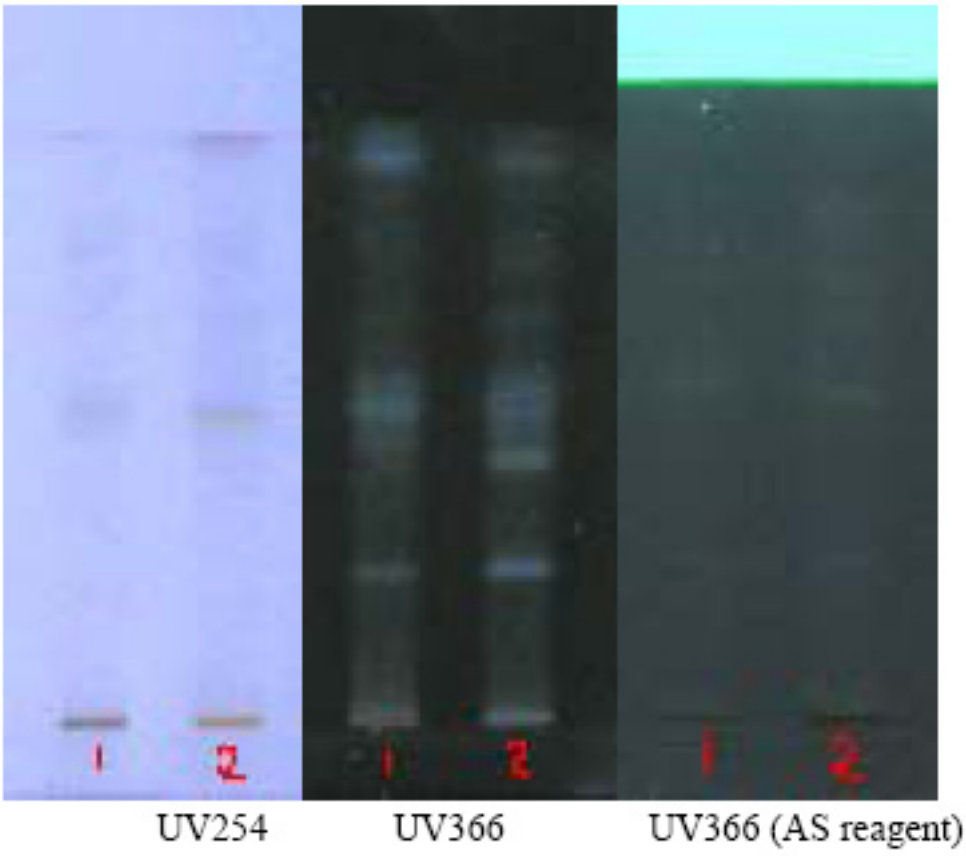

Figure 1. HPTLC fingerprint of both formulations (methanol extract) *Key: 1= methanol extract of Efiplus caps® 2= methanol extract of HMF-TE AS reagent- Anisaldehyde- sulphuric acid acid reagent.*

In the present study it was found that HMF-TE double dose has the faster effect in rats upon the selected parameters haemoglobin, haematocrit, RBCs, MCV, MCH.

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