Comparative Study of Femineral[®] and Floradix[®] in Women of Child-Bearing Age and Adolescent Girls with Iron Deficiency Anaemia

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Abstract

This double blind randomised trial compared the clinical efficacy of Femineral[®], a proprietary herbal preparation containing highly soluble iron compounds, minerals, and vitamins, with Floradix[®], the most popular iron supplement in Scandinavia for patients with iron deficiency anaemia (IDA).

Fifty women of childbearing age and adolescent girls, all diagnosed with IDA, were randomised into two groups. One group (n = 26) was treated with Femineral (10 ml twice a day; 15 mg of iron supplement/day), while the second (n = 24) received Floradix[®] (10 ml twice a day; 20 mg of iron supplement/day). The efficacy of 28 days of treatment in each group was evaluated by analysis of serum iron levels, blood haemoglobin and erythrocyte counts; by the mental performance of the patients on days one and 28; and by assessment of IDA symptoms, such as pale skin colour, fatigue, irritability, weakness, constipation, brittle nails, cold hands and feet, headache, blue tinge to sclera, and feeling of well being on days one, seven, 14 and 28.

The Femineral group showed significant improvements in blood haemoglobin levels and red cell counts after 28 days, while the Floradix group did not. The

Femineral group showed better improvements in other IDA symptoms, such as impaired mental performance, pale skin colour, fatigue, irritability, weakness, constipation, brittle nails, cold hands and feet. Both groups showed significant improvements in serum iron levels at the end of the study. Such improvements correlate with the results of memory tests: the number of errors by both groups was significantly lower at the end of the study. There were no significant differences in memory tests between the two groups. Eight patients in the Floradix group and one in the Femineral group reported drowsiness. The Femineral group reported two other adverse events (nausea and vomiting). Femineral can be used safely in the treatment of iron deficiency anaemia. It is more efficient and safe than Floradix.

Keywords

Herbal preparations • Iron deficiency and anaemia • Clinical trial

Introduction

Iron deficiency anaemia (IDA) is the most ubiquitous nutritional deficiency worldwide, and is particularly prevalent in women and infants [1, 2]. The processes of menstruation, pregnancy, and breast-feeding place great strains on iron stores, so that women of childbearing age most commonly suffer from low blood-iron levels. It is reported that while 3% of men present iron deficiency, around 20% of all women, and 50% of pregnant women suffer from this condition [3].

Iron is essential for the formation of haemoglobin, and for the effective circulation of oxygen throughout the body. An inadequate level of iron in the blood induces fatigue, reduces physical activity, and affects behavior, psychomotor development, and scholastic performance [4–6]. Iron status is a significant factor in the cognitive performance in women of reproductive age. Severe anaemia primarily affects processing speed, and severe iron deficiency affects accuracy of cognitive function over a broad range of tasks [7]. In pregnancy, IDA is associated with an increased risk of pre-term delivery, low birth weight, and higher incidences of maternal mortality [8, 9].

Reliance on dietary intake to provide an appropriate amount of iron is complicated because the various types of iron present in different foods exhibit somewhat different characteristics. The bio-availability of the element may also depend on the presence of other dietary components. Thus, heme iron from animal products has a better availability and absorption (10% – 25%) compared with that of non-heme iron (3% – 8%), and the simultaneous consumption of vitamin C can increase the absorption rate of available iron by a factor of four [10].

One of the most frequently recommended strategies for improving iron status in vulnerable groups involves iron supplementation [11]. Iron supplementation improves verbal learning and memory in non-anaemic iron-deficient adolescent girls [12]. However, increasing the amount of iron, which can enter the intestinal tract in an unabsorbed form, may result in various side effects, including constipation, allergy, yeast-sensitivity, and liver disorders. These side effects are problems of concern with respect to most iron supplements [13].

The aim of the present study is to compare the efficacy and safety of Femineral® (Swedish Herbal Institute, Gothenburg, Sweden), a new proprietary dietary supplement designed for patients who suffer from IDA, with Floradix® (Salus-Haus, Bruckmuhl, Germany) [14], the most popular iron supplement in Scandinavia for patients with IDA. Femineral contains highly soluble iron compounds, combined with vitamins B1, B2, B6, B9 and B12, vitamin C, minerals, and digestion-enhancing herbs.

Experimental

Study design

A comparative, randomised, double-blind, parallel-group trial with positive control. The sample population comprised 50 patients, including women of childbearing age and adolescent girls, all of whom had been diagnosed with IDA. The study was carried out in accordance with the principles of Good Clinical Practice (GCP) at the Department of Clinical Pharmacology, Armenian National Institute of Health, Yerevan, Armenia, during the spring of 2007. Under the direction of the investigator and the trial monitor, the protocols of the study were presented to, reviewed and approved by the Ethics Committee of the Armenian Scientific Centre of Drug and Medical Technology Expertise of the Ministry of Health of the Republic of Armenia. In compliance with the revised declaration of Helsinki [15], written informed consent was obtained from each participant prior to inclusion in the trial.

Adolescent girls and women of childbearing age who were attending the Yerevan City Polyclinic No. 8, Yerevan, Armenia, and showing symptoms of IDA were considered for inclusion in the trial. Potential participants initially underwent blood analysis (serum iron, erythrocyte and haemoglobin count) and an examination by a physician in order to confirm the diagnosis of IDA. The criteria for inclusion in the study were: healthy girls and fertile women; ages between 14 and 50 years; regular menstruation in the last three months; characteristic clinical symptoms of IDA, including a serum ferritin level less than 12 mg/l, serum iron level less than 70 µg/dl and blood haemoglobin level less than 120 g/l. The criteria for exclusion were: use of antibiotics, multivitamins, mineral supplements or gastric acidity-increasing preparations in the last six months; or regular consumption of liver.

Sample size: Assuming a 30% effect difference between the treatment group and the placebo group between points in time with a 95% confidence interval and a maximal drop-out of 20% a sample size of (2 x 25) was calculated as sufficient for a (1–5%) significance level [16]. The patients were evaluable according to the intention-to-treat principle. No patient was excluded due to medical noncompliance or adverse drug reactions.

Study preparations

The test preparations Femineral and Floradix-Blutsaft were manufactured in liquid form according to Good Manufacturing Practice (GMP) by the Swedish Herbal Institute and by Salus-Haus, respectively. Their declared contents are shown in Table 1. All preparations were produced with identical organoleptic appearance (colour, taste and smell), and were indistinguishable from each other. Preparations were provided in dark glass bottles (500 ml) with a cap, sealing ring, and a measuring dosage cup (graduated at 5, 10, 15, 20 and 30 ml), and labelled “Femineral/Floradix 500 ml: Batch Number..., Clinical Study, Dose 10 ml – 2-times a Day, Patient Number...”. The identification number on each of the bottles

was randomly encoded in a preparation list so that the nature of the preparations could be ascertained after completion of the study in order to perform the statistical analyses. The filling and labelling of preparation bottles was performed in the presence of a pharmacist, and all preparations were stored separately at room temperature in a secure location. Daily dose of iron: 20 mg in 20 ml of Floradix, and 15 mg in 20 ml of Femineral.

Tab. 1. Formulation of the preparations Floradix and Femineral employed in the trial.

Active Ingredients of Femineral		g/100 mL
<i>Angelica archangelica</i> , root 50% ethanol extract, DER 15:1		0.5
<i>Astragalus membranaceus</i> , herb 50% ethanol extract, DER 4,36:1		1.67
<i>Eleutherococcus senticosus</i> , root 70% ethanol extract, DER 10,2:1		5
<i>Nepeta cataria</i> , herb 50% ethanol extract, DER 2,7:1		2.0
<i>Verbena officinalis</i> , herb 50% ethanol extract, DER 4,45:1		4.17
50% ethanol extract of fixed combination of the herbal mixture, DER 0,66:1		3.3
<i>Achilea millefolium</i> , herb, 3.4%		0,113
<i>Betula pendula</i> , leaf, 13.6%		0,453
<i>Calendula officinalis</i> , flowers, 6.8%		0,226
<i>Citrus auranticum</i> , fruit peel 3,4%		0,113
<i>Crategus oxyacantha</i> fruit, 3.4%		0,113
<i>Equisetum arvense</i> , herb, 13.6%		0,453
<i>Graminis repens</i> , rhizome, 1,4%		0,047
<i>Hibiscus sabdariffa</i> , flowers, 3.4%		0,113
<i>Hyssopus officinalis</i> , herb, 6.8%		0,226
<i>Medicago sativa</i> , herb, 17%		0,566
<i>Ribes cynosbati</i> . fruit, 3.4%		0,113
<i>Rosmarinus officinalis</i> , leaf, 6.8%		0,226
<i>Urtica dioica</i> , leaf, 17%		0,566
Tiamin HCl – Vitamin B ₁		0,02
Riboflavin Na- Vitamin B ₂		0,02
Pyridoxin HCl - Vitamin B ₆		0,024
Cyanocobalamin - Vitamin B ₁₂		0,00005
Folic acid – h		0,002
Ascorbic acid		0.1
Iron-gluconate	Equivalent to 75 mg Fe	0.577
Zinc-gluconate	Equivalent to 50 mg Zn	0,39
Copper-gluconate	Equivalent to 8.33 mg Cu	0,06
Manganese-gluconate	Equivalent to 12.5 mg Mn	0,113
Sodiumiodide	Equivalent to 625 µg I	0,00073
Vanadiumsulfate	Equivalent to 416 µg V	0,00022
Ammonium-molybdenum	Equivalent to 625 µg Mo	0,00117
ChromeMate	Equivalent to 208 µg Cr	0,005
Sodiumselenite	Equivalent to 167 µg Se	0,000555
Boron citrate	Equivalent to 104 µg B	0,000002
Active Ingredients of Floradix		g/100 mL
<i>Achilea millefolium</i> , herb, extract		Not specified
<i>Angelica archangelica</i> , root extract		Not specified

Tab. 1. (Cont.)

<i>Artemisia absinthium</i> , herb extract	Not specified
<i>Beta vulgaris</i> , root extract	Not specified
Cascarilla <i>linearis</i> , bark extract	Not specified
<i>Citrus auranticum</i> , peel extract	Not specified
<i>Daucus carota</i> , root extract	Not specified
<i>Eleutherococcus senticosus</i> , root extract	Not specified
<i>Eqiusetum arvense</i> , herb, extract	Not specified
<i>Foeniculum vulgare</i> , fruit, extract	Not specified
<i>Hibiscus sabdariffa</i> , flowers extract	Not specified
<i>Phaeophyceae</i> , brown alga, extract	Not specified
<i>Spinacia oleracea</i> , leaf, extract	Not specified
<i>Urtica dioica</i> , leaf extract	Not specified
<i>Citrus sinensis</i> , jouce	Not specified
<i>Prunus Serotina</i> , jouce	Not specified
<i>Pyrus communis</i> , jouce	Not specified
<i>Ribes nigrum</i> , jouce	Not specified
<i>Rubus fruticosus</i> , jouce	Not specified
Vitis vinifera , jouce	Not specified
Tiamin HCl – Vitamin B ₁	0.007
Riboflavin Na- Vitamin B ₂	0.008
Pyridoxin HCl - Vitamin B ₆	0.0075
Cyanocobalamin - Vitamin B ₁₂	0.000001
Ascorbic acid	Not specified
Iron-gluconate Equivalent to 100 mg Fe	0.770

Randomization and blinding

During their first contact with the project physician, patients selected for inclusion in the trial were divided into two groups (A and B) by a process of simple randomisation. Each participant received an identification number between one and 50, allocated in strict order of treatment inception, together with a bottle number (randomly assigned by the investigator), an individual test-book, an analysis log, and a health diary. The correlation between bottle number, preparation, and group was contained in a list in which the random (casual) distribution of labelled test samples (Femineral or Floradix) had been encoded prior to the start of the trial. This list was not available to investigators, patients, or care-providers, all of whom were thus fully blinded with respect to treatment assignment until the statistical analyses were completed at the end of the trial. For each patient, the casually-obtained identification number together with the code number of the randomly assigned bottle containing the preparation were recorded in a protocol, in the self-assessment journal of the patient, and on a clinical report form (CRF). The key to the code (related to assignment groups A and B to Floradix ore Femineral treatment) was maintained secure by the project monitor and only provided to statistician after the results were evaluated statistically.

Methodology, data collection, and efficacy parameters

An investigator completed a baseline questionnaire for each patient after randomization, recording age and a detailed history of illnesses. Additional information concerning skin colour, fatigue, irritability, weakness, constipation, brittle nails, cold hands and feet, headache (frontal), blue tinge to sclera, and general well being, was gathered by the investigator for each patient. Patients gave self-assessments of these symptoms using visual analogue scales (VAS) for each one. The endpoints of the scales were marked at 0 cm (representing pronounced problems) and at 10 cm (representing no problems). The responses to the self-assessment questionnaire were recorded by the principal investigator with the assistance of the patient, and entered into the patient's health diary. The results of these surveys were collected in the CRFs on day one, and on days seven, 14, and 28.

In order to evaluate functional components of each participant's mental activity, including the capacity of short-term memory, the stability and intensity of attention, and the speed of mental performance, the principal investigator then administered a validated psychometric test ("arrangement of numbers") [17] to each patient in an isolated, noiseless room. Mental performance tests on day one, and on day 28, were performed in the afternoons between 13.00 and 15.00 hours. The investigator presented a square of 25 cells containing numbers in a random arrangement to each of the participants, along with a blank square of 25 cells. A strict time limit of two minutes (measured using a stop-watch) was allowed for completion of the test. The results were checked against a key and the count of numbers arranged in the correct squares (showing productivity and speed of work) and the count of omitted numbers (showing errors and accuracy of work) were recorded in the participant's CRF on day one, and on day 28.

After being grouped, and having completed the baseline measures, the patients immediately commenced a 28-day treatment, with group A ($n = 24$) receiving Floradix (10 ml dose given orally twice a day, totaling a 20 mg daily dose of iron), and group B ($n = 26$) receiving Femineral (10 ml dose given orally twice a day, totalling a 15 mg daily dose of iron). Blood used for standard laboratory analysis of the concentration of circulating iron that is bound to transferrin (serum iron), haemoglobin (g/L) and the erythrocytes (in $10^{12}/L$) was collected in the morning before breakfast on day one, and on day 28.

The patients were visited to the physician for clinical examinations at day one, seven, 14 and 28. Also evaluation of memory test and laboratory analysis was performed at day one and 28.

The daily health assessment was performed by patients and fill in the Self-Assessment Journal every day in the evening at 20.00–22.00.

Monitoring the study

The trial was monitored by an independent monitor in accordance with the guidelines for GCP of the International Conference on Harmonisation [18] and the Commission of the European Communities [19]. The monitor visited the study centre every second week, and telephone contacts were made every week. The aims of the monitor were to review the daily status of the study, to verify adherence to the study protocol, to ensure the completeness and accuracy of the CRFs and their agreement with the source documents.

Adequate time was reserved for each visit of the monitor in which the CRFs could be examined on site and any necessary corrections could be made immediately.

Patient safety and compliance

Patients were informed at the start of the study that they should cease treatment immediately and seek immediate contact with the investigator should any concurrent illness or side effects develop while taking the preparation. Additionally, each patient was provided with a sealed, emergency code envelope containing information specifying which treatment the patient was receiving during the trial. These envelopes were stored under control of the Coordinator of the study. Patient compliance was determined by questioning the patients and by collecting the bottles of preparation with unused contents during the final visit to the physician. The volume of unused liquid was measured against the upper limit for compliance, which had been set at 2%.

Statistical methods

All data relevant to the study were entered patient by patient into the CRF database (Microsoft[™] Excel[®] 2000) according to each patient's identification number. The mean, standard error of the mean, and standard deviation (SD) values for the VAS scores were calculated according to standard methods of statistical analysis.

In order to ascertain whether the mean values of the parameters determined in the two groups of patients at the beginning of the treatment were statistically significant, unpaired t-tests (two-tailed) and Mann-Whitney tests were applied. For the comparison of the data associated with the efficacy of the treatment collected from each group of patients on days one, seven, 14 and 28, one-way repeated ANOVA and Bonferroni's multiple comparison post-tests were applied. In order to evaluate the difference between the efficacies of the treatments at the end of the study, the means of the differences between the beginning and the end of the treatment were compared for each variable by application of one-way analysis of variance with Bonferroni's multiple comparison post-tests.

Two statisticians independently performed data management and calculations using GraphPad (San Diego, CA, USA) Prism software (version 3.03 for Windows) and Statistica 6.0 (Statsoft, Tulsa, OK, USA) statistical software.

Results

The study population of 50 women and adolescent girls, ages between 14 and 50 years (mean age 35.54 ± 12.20 years) and presenting IDA, was randomly distributed between group A ($n = 24$; mean age 34.75 ± 12.13 years; treated with 20 ml Floradix per day) and group B ($n = 26$; mean age 36.27 ± 12.46 years; treated with 20 ml Femineral per day). No statistically significant differences were observed between the two groups with respect to the baseline levels of serum iron, haemoglobin and erythrocytes (see Table 2-4), mental performance tests (see Table 5), and other IDA symptoms (see Table 6) determined at the start (day one) of the study.

Tab. 2. Improvement in parameters following taking of Femineral or Floradix as indicated by the results of serum iron ($\mu\text{g/dL}$) determined on the first and last days of the 28 day trial.

Groups Patients	Floradix –Group A			Femineral – Group B		
	1st day-A	28 th day-A	Difference	1 st day -B	28 th day-B	Difference
1	68,6	78,6	10,0	64,8	92,9	28,1
2	31,3	72,6	41,3	57,1	80,0	22,9
3	60,4	80,2	19,8	69,4	79,8	10,4
4	70,2	110,8	40,6	61,4	82,6	21,2
5	68,2	81,2	13,0	60,2	89,3	29,1
6	58,5	100,0	41,5	69,0	82,0	13,0
7	65,8	82,3	16,5	61,9	78,3	16,4
8	60,4	90,2	29,8	54,8	110,2	55,4
9	37,4	72,4	35,0	68,0	92,8	24,8
10	60,2	75,0	14,8	70,0	105,0	35,0
11	70,0	92,0	22,0	65,0	85,0	20,0
12	47,2	68,4	21,2	58,0	80,0	22,0
13	69,6	80,4	10,8	66,8	100,0	33,2
14	33,0	68,0	35,0	25,6	58,0	32,4
15	53,0	78,0	25,0	60,0	96,0	36,0
16	60,0	80,0	20,0	60,2	96,0	35,8
17	32,4	70,2	37,8	58,4	80,0	21,6
18	27,3	58,4	31,1	10,0	58,2	48,2
19	55,2	78,5	23,3	64,3	100,5	36,2
20	36,8	80,5	43,7	58,4	89,3	30,9
21	48,5	67,0	18,5	24,5	83,7	59,2
22	54,5	80,4	25,9	59,2	92,0	32,8
23	58,2	72,8	14,6	64,0	100,0	36,0
24	42,8	60,5	17,7	60,4	80,2	19,8
25				34,2	65,4	31,2
26				52,8	72,4	19,6
Mean	52,90	78,27	25,37	56,09	85,75	29,66
Std. Dev	13,82	11,65	10,59	15,20	13,17	11,81
Compar- ison	1 st day-A – 28 th day A		1 st day A – 1 st day B	1 st day-B – 28 th day B		28 th day A – 28 th day B
Mean Diff	25,37		3.196	29,66		4.291
t	6.851		0.8803	8.337		1.182
P value	<0.001		>0.05	<0.001		>0.05

Tab. 3. Improvement in parameters following taking of Femineral or Floradix as indicated by the results of blood haemoglobin (g/L) determined on the first and last days of the 28 day trial.

Groups Patients	Floradix – Group A			Femineral – Group B		
	1st day-A	28th day-A	Difference	1st day -B	28th day-B	Difference
1	120,0	140,0	20,0	110,0	117,0	7,0
2	100,0	120,0	20,0	120,0	134,0	14,0
3	110,0	110,0	0,0	120,0	130,0	10,0
4	110,0	120,0	10,0	100,0	110,0	10,0
5	120,0	136,0	16,0	99,0	110,0	11,0
6	90,0	100,0	10,0	99,0	110,0	11,0
7	120,0	120,0	0,0	99,0	110,0	11,0
8	110,0	112,0	2,0	120,0	140,0	20,0
9	100,0	110,0	10,0	99,0	110,0	11,0
10	110,0	110,0	0,0	116,0	130,0	14,0
11	110,0	110,0	0,0	120,0	130,0	10,0
12	100,0	100,0	0,0	99,0	120,0	21,0
13	90,0	100,0	10,0	99,0	110,0	11,0
14	110,0	112,0	2,0	60,0	90,0	30,0
15	90,0	90,0	0,0	110,0	120,0	10,0
16	110,0	110,0	0,0	90,0	110,0	20,0
17	110,0	112,0	2,0	112,0	122,0	10,0
18	100,0	110,0	10,0	90,0	110,0	20,0
19	100,0	100,0	0,0	93,0	100,0	7,0
20	100,0	100,0	0,0	100,0	120,0	20,0
21	110,0	112,0	2,0	90,0	110,0	20,0
22	110,0	112,0	2,0	90,0	112,0	22,0
23	99,0	100,0	1,0	90,0	120,0	30,0
24	102,0	110,0	8,0	100,0	120,0	20,0
25				96,0	110,0	14,0
26				100,0	100,0	0,0
Mean	105,5	110,7	5,208	100,8	115,6	14,77
Std. Dev	8,822	11,20	6,561	13,20	11,25	7,056
Comparison	1 st day-A – 28 th day A		1 st day A – 1 st day B	1 st day-B – 28 th day B		28 th day A – 28 th day B
Mean Diff	5,208		4.651	14,77		9,561
t	1.802		1.641	5,32		3,374
P value	>0.05		>0.05	<0.001		<0.05

Tab. 4. Improvement in parameters following taking of Femineral or Floradix as indicated by the results of blood erythrocytes ($10^{12}/L$) determined on the first and last days of the 28 day trial.

Groups Patients	Floradix –Group A			Femineral – Group B		
	1st day-A	28 th day-A	Difference	1 st day -B	28 th day-B	Difference
1	3,8	3,8	0,0	3,8	3,8	0,0
2	3,2	3,6	0,4	3,6	3,8	0,2
3	3,6	3,6	0,0	4,0	4,0	0,0
4	3,8	3,8	0,0	3,8	3,8	0,0
5	4,0	4,3	0,3	3,6	3,8	0,2
6	3,6	3,8	0,2	3,6	3,8	0,2
7	3,6	3,6	0,0	3,6	3,8	0,2
8	3,6	3,8	0,2	3,6	3,6	0,0
9	3,2	3,2	0,0	3,6	3,9	0,3
10	3,2	3,4	0,2	3,9	4,0	0,1
11	3,6	3,6	0,0	3,8	4,0	0,2
12	3,2	3,4	0,2	3,8	4,0	0,2
13	3,2	3,2	0,0	3,6	3,8	0,2
14	3,6	3,6	0,0	1,8	2,6	0,8
15	2,8	3,2	0,4	3,4	3,8	0,4
16	3,6	3,6	0,0	3,2	3,8	0,6
17	3,6	3,6	0,0	3,6	3,8	0,2
18	3,2	3,6	0,4	3,2	3,6	0,4
19	3,2	3,2	0,0	2,8	3,2	0,4
20	3,6	3,6	0,0	3,6	3,8	0,2
21	3,6	3,6	0,0	3,2	3,6	0,4
22	3,6	3,6	0,0	3,2	3,6	0,4
23	3,4	3,4	0,0	3,4	3,8	0,4
24	3,4	3,4	0,0	3,6	3,8	0,2
25				3,2	3,6	0,4
26				3,6	3,6	0,0
Mean	3,467	3,563	0,09583	3,465	3,719	0,2538
Std. Dev	0,2681	0,2481	0,1488	0,4335	0,2857	0,1944
Comparison	1 st day-A – 28 th day A		1 st day A – 1 st day B	1 st day-B – 28 th day B		28 th day A – 28 th day B
Mean Diff	0,09583		0,00128	0,2538		0.158
t	1.187		0,01620	3,273		1.996
P value	>0,05		>0.05	<0.05		>0.05

Tab. 5. Improvement in parameters following taking of Femineral or Floradix as indicated by the difference in the total number of errors in mental performance test determined on the first and last days of the 28 day trial.

Groups Patients	Floradix –Group A			Femineral – Group B		
	1 st day-A	28 th day-A	Difference	1 st day -B	28 th day-B	Difference
1	6,0	1,0	5,0	13,0	2,0	11,0
2	7,0	1,0	6,0	12,0	6,0	6,0
3	11,0	4,0	7,0	14,0	5,0	9,0
4	12,0	5,0	7,0	11,0	2,0	9,0
5	11,0	2,0	9,0	4,0	2,0	2,0
6	3,0	0,0	3,0	12,0	4,0	8,0
7	16,0	11,0	5,0	4,0	0,0	4,0
8	16,0	8,0	8,0	13,0	7,0	6,0
9	12,0	4,0	8,0	11,0	5,0	6,0
10	1,0	0,0	1,0	1,0	0,0	1,0
11	11,0	4,0	7,0	5,0	1,0	4,0
12	11,0	3,0	8,0	7,0	2,0	5,0
13	1,0	0,0	1,0	1,0	0,0	1,0
14	13,0	5,0	8,0	4,0	1,0	3,0
15	18,0	9,0	9,0	14,0	3,0	11,0
16	11,0	3,0	8,0	13,0	5,0	8,0
17	11,0	4,0	7,0	6,0	4,0	2,0
18	2,0	0,0	2,0	7,0	2,0	5,0
19	5,0	1,0	4,0	8,0	2,0	6,0
20	5,0	1,0	4,0	4,0	1,0	3,0
21	4,0	2,0	2,0	6,0	2,0	4,0
22	9,0	3,0	6,0	9,0	1,0	8,0
23	11,0	2,0	9,0	9,0	1,0	8,0
24	9,0	2,0	7,0	8,0	2,0	6,0
25				6,0	0,0	6,0
26				7,0	3,0	4,0
Mean	9,000	3,125	5,875	8,038	2,423	5,615
Std. Dev	4,773	2,894	2,576	3,924	1,943	2,815
Comparison	1 st day-A – 28 th day A		1 st day A – 1 st day B	1 st day-B – 28 th day B		28 th day A – 28 th day B
Mean Diff	5,875		0,9615	5,615		0.2596
t	6,21		1,036	6,178		0,2798
P value	<0,001		>0.05	<0.001		>0.05

Tab. 6. Within-group improvement in symptoms following tacking of Femineral or Floradix as indicated by the difference in VAS scores determined on the first and last days of the 28 day trial.

Symptoms	Day 1 (baseline level) [†]		Day 7		Day 14		Day 28	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group A (n = 24) treated with Floradix								
Skin colour	1.75	0.94	2.45	0.97***	3.46	0.77****	5.41	0.77****
Fatigue	1.83	1.04	2.46	0.97***	3.46	0.78****	5.42	0.77****
Irritability	2.66	1.27	3.17	1.05**	3.92	0.88***	5.66	1.01****
Weakness	2.96	1.36	3.62	1.38**	4.54	1.06****	5.87	0.94****
Constipation	3.21	1.84	4.62	1.61***	5.58	1.44***	6.62	1.05****
Brittle nails	1.46	0.51	2.37	0.87***	3.71	0.81****	5.7	0.8****
Cold hands and feet	2.54	1.35	3.71	1.33***	5.04	0.95****	6.33	0.81****
Headache (frontal)	3.37	1.34	4.17	1.27**	5.54	0.93****	6.83	0.96****
Blue tinge to sclera	5.2	1.1	5.58	0.97*	6.33	0.87***	7.25	0.53****
Well being	4.42	1.1	5.25	1.07***	6.33	0.76****	7.62	0.49****
Group B (n = 26) treated with Femineral								
Skin colour	1.61	0.69	3.11	0.51****	5.0	0.8****	7.88	0.86****
Fatigue	1.65	0.63	3.23	0.51****	5.23	1.07****	7.88	0.86****
Irritability	2.2	0.89	3.61	0.69****	5.19	0.75****	8.0	0.74****
Weakness	2.73	1.18	4.11	0.99****	5.65	0.89****	8.3	0.78****
Constipation	3.11	1.72	4.76	1.27****	6.31	0.88****	8.46	0.81****
Brittle nails	1.53	1.42	2.81	1.41****	4.81	1.23****	7.96	0.82****
Cold hands and feet	2.11	1.1	3.92	0.97****	5.69	0.83****	8.57	0.85****
Headache (frontal)	2.69	1.15	4.38	0.89****	6.15	0.83****	9.03	0.72****
Blue tinge to sclera	4.76	1.39	5.73	1.34****	6.84	0.88****	9.03	0.66****
Well being	3.92	0.97	5.5	0.98****	6.96	0.59****	9.46	0.76****

[†] There were no significant differences ($P > 0.05$) between groups A and B with respect to the baseline (day 1) values of the individual VAS scores.

[§] The significances of differences between groups are indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

No patients withdrew or dropped-out during the period of the trial, and at the end of the study all 50 patients had completed daily health records. The correlation between the amount of unused preparation returned and the information revealed by each patient concerning the number of days that preparation had been administered was extremely high, showing that all participants had fulfilled the compliance criterion by taking at least 98% of the total recommended dose.

Only one patient (3.8%) reported drowsiness after administration of Femineral, while a significantly ($p < 0.05$) larger percentage (33.3%; 8 patients) suffered from this condition following treatment with Floradix. During the first two to four days of treatment with Femineral, one patient (3.8%) complained of nausea and one (3.8%) of vomiting (values that were not statistically non-significant), although no further adverse drug reactions were reported after the fourth day of treatment with Femineral, and none were reported following treatment with Floradix.

The overall efficacies of the treatments applied were assessed by statistical analyses of the differences between the mean values determined on the first and last days with respect to the primary endpoints – the levels of serum iron, blood haemoglobin and erythrocytes (Tables 2–4), as well as to the secondary endpoints, such as mental performance (Table 5) and the each of the subjective IDA symptoms (Table 6). Significant improvement in blood iron level was observed in both groups of patients (Table 2). The difference between the two groups at the end of the treatment was not significant (Table 2). Femineral treatment was more efficient, as the daily consumption of iron from Femineral was less than from Floradix (15 mg vs. 20 mg). Significant improvements in blood haemoglobin levels and red blood cell counts were observed at the end of the study in Femineral group, while these levels were not improved in the group of patients taking Floradix (Tables 3 and 4). The comparison of the two groups at the end of the study shows significant difference in favour of Femineral (Table 3).

The improvements in serum iron levels correlate with improvements in the memory results in the psychotropic tests of mental performance (Table 5). The number of errors was significantly lower at the end of the study in both groups of patients. There were no significant differences between the mental performance test results of the Femineral and the positive control group of patients.

Improvements in other IDA symptoms were observed by the physician and expressed in the VAS scores. The validity of evaluations by physical examinations of patients and by VAS scores is uncertain. However, they may be taken into account as adjuvant tools for evaluation the efficacy of treatment. VAS scores were improved for all symptoms in both groups starting from day seven of treatment (Table 6). Assuming that the impact of each variable has equal weighting, the sum of total VAS scores is shown on the figure 1. The enhancement of the total mean VAS scores after seven, 14 and 28 days of treatment was more rapid in the Femineral group than in the Floradix group.

Fig. 1. Total mean VAS scores ($\pm$ SD) of iron deficiency anemia symptoms (secondary endpoints) measured on days 1 (start), 7, 14 and 28 (end) of the trial of Floradix (group A) and Femineral (group B). Within each group, the significance of the difference between each mean value and that measured on day 1 is shown as ^{ns} not significant ($P \geq 0.05$) and significant at ** $P < 0.001$ and *** $P < 0.0001$.

Discussion

This study shows that the Femineral is more efficient for the treatment of IDA than the most popular iron supplement in Scandinavia, Floradix. Femineral is superior to Floradix in improving blood haemoglobin level. It is difficult to give the rational explanation for this finding, however, we hypothetically speculate that it may be due to the herbal extracts or microelements present in Femineral. That is to say that the differences in efficacies observed between Floradix and Femineral may be associated with the fixed amounts of trace elements contained in the latter. Deficiencies in selenium, molybdenum, chromium, boron can give rise to IDA. Moreover, deficiency in manganese (a key element in enzyme systems responsible for protein synthesis) is often associated with iron deficiency, while copper deficiency should be considered in all patients presenting unexplained anaemia, neutropenia or myelodysplasia [20]. The inclusion of copper gluconate in Femineral improves iron absorption, while the addition of zinc can increase haemoglobin levels more than iron alone can do [21]. The simultaneous occurrence of Zn and Fe deficiencies in humans has long been known, and it has been recently been demonstrated that low Fe stores *per se*, or Fe-deficiency anaemia, imply low Zn status [22]. Additionally, Femineral contains folic acid (vitamin B11), which is a coenzyme in the biosynthesis of haemoglobin [23], and also assists in the prevention of pernicious anaemia.

The herbal extracts present in Femineral may also impart beneficial effects in anaemia. The juice of the stinging nettle (*Urtica dioica*) is rich in both iron and vitamin C (which is thought to promote the absorption of iron); the root of angelica species stimulates the circulatory system and aids digestion, while astragalus species, calendula, *Medicago*,

Nepeta, *Verbena*, *Crategus*, *Hissopus*, *Rosmarinus* and other herbal extracts of Femineral are used in alternative and traditional medicines to relieve iron deficiency induced symptoms [24–28].

The present study demonstrates that a four-week treatment with the proprietary preparation Femineral significantly increases hemoglobin and serum iron levels, and erythrocyte counts, with the concomitant reduction or elimination of symptoms of iron deficiency and anaemia including pale skin colour, fatigue, irritability, weakness, constipation, brittle nails, cold hands and feet, headache, blue tinge to sclerae, low feeling of well being, and memory impairment. Furthermore, it shows that treatment with Femineral is significantly more efficient than treatment with Floradix, even though the daily consumption of iron is lower with the former preparation (15 mg/day for Femineral *versus* 20 mg/day for Floradix).

Conclusions

Femineral can be used effectively and safely for the treatment of iron deficiency anaemia as the iron supplement Floradix. Femineral is significantly more efficient with respect to erythrocytes and haemoglobin than Floradix, well tolerated and safe.

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Authors' Statements

Competing Interests & Authors' Contributions

The study was conceived by GW, the Director and a stockholder of SHI Research and Development. The funding sponsor, however, had no role in any practical aspect of the study including experiments, data collection, management, analysis and interpretation of the data. The study protocol and ethical application was formulated by GA. GB enrolled patients and performed the study interventions, assays, and data analysis: further data analyses were performed by EA. AP. drafted the manuscript, and all authors were involved in its critical appraisal and final approval. AP is employed by the Sponsor (SHI) on a permanent basis. GB receives an honorarium from the sponsor for contract research carried out on behalf of SHI. EG and EA have no commercial associations or financial interests with respect to this study.

Informed Consent & Ethical Approvals

The institutional and (inter)national ethical guides for experiments on human subjects were followed and informed consent was obtained. See the experimental part for details.

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