



International Journal of Indian Medicine

www.ijim.co.in

ISSN: 2582-7634

Volume - 6, Issue - 4



INDEXED

April 2025



International Journal of Indian Medicine



International Category Code (ICC): ICC-1702 International Journal Address (IJA): IJA.ZONE/258276217634 eISSN : 2582 - 7634

A Single Arm Clinical Trial to Evaluate Harithakiyogam as Tablet Form in Iron Deficiency Anaemia

Lavanya G J¹, Deepa M.S.²

1. Former MD Scholar, Department of Dravyaguna Vigyana, Govt. Ayurveda College Trivandrum
2. Professor and HOD, Department of Dravyaguna Vigyana, Govt. Ayurveda College Trivandrum

Abstract:

Background: Iron Deficiency Anaemia (IDA) constituting 66.2% of total anaemia cases, with 825 million women and 444 million men affected globally. Major symptoms are reduced appetite, dryness of skin, fatigue, weakness, dyspnoea on exertion, palpitation & pallor of the skin, mucous membranes & sclera etc. Several Iron contained medicines are conventionally used for the management of IDA whereas some of them have adverse reactions such as nausea, heartburn, constipation etc. Hence usage of safe and effective alternative correction of basic pathology of disease is to be taken. **Objective:** Primary objective is to find out the effectiveness of ayurvedic formulation termed as Harithakiyogam in Iron deficiency Anaemia. **Materials and Methods:** It is designed as quasi-interventional study. Total 23 participants of both male and female of age group 18-50 years having Haemoglobin level 8-12.9 g/dl for male and 8-11.9 g/dl for female were selected by consecutive sampling method. Participants were advised to take 500 mg of 1 tablet thrice daily after food for 3 months. Outcome parameters were Change in Haemoglobin level, red cell indices, Peripheral smear, RDW-CV and monitored on 0th day and 91st day. The obtained data were statistically analysed using Paired 't' test. **Results:** After clinical trial, outcome parameters have statistically significant improvement ($p < 0.01$) after the intervention period. Clinical symptoms were also significantly improved after the trial. **Conclusion:** The Trial drug was found effective in improving haemoglobin, red cell indices, microcytic hypochromic cells and RDW-CV value and clinically significant in reducing the associated symptoms.

Keywords: Iron deficiency Anaemia; Microcytic hypochromic; RDW-CV.

Corresponding Author:

Lavanya G J

Former MD Scholar, Department of Dravyaguna Vigyana,
Govt. Ayurveda College Trivandrum

Email- lavanyagj96@gmail.com

How to cite this article:

Lavanya G J, Deepa M.S. A Single Arm Clinical Trial to Evaluate Harithakiyogam as Tablet Form in Iron Deficiency Anaemia. Int J Ind Med 2025;6(4):01-12 DOI: <http://doi.org/10.55552/IJIM.2025.6401>

INTRODUCTION:

Iron deficiency anaemia arises when the iron supply is insufficient to meet the needs of haemoglobin production. Its prevalence as per the National Family Health Survey 5 (2019-21), is 59.1 percent in adolescent girls, 31.1 percent in adolescent boys (15-19 yrs), 57.0 percent in women (15-49 years) and 25.0 percent in men (15-49 years).^[1] Clinical signs of iron deficiency anaemia include oedema, weakness, anorexia, pallor, and irritability, pica etc. Several Iron contained medicines are conventionally used for the management of IDA whereas some of them have adverse reactions such as constipation, abdominal cramps, nausea, vomiting, diarrhoea etc.^[2] IDA is managed with so many formulations mentioned under *Panduroga chikitsa* (Treatment of pallor/clinical features of pāṇḍu) in Ayurveda also. *Harithakiyogam* is one among such formulation which has limited number of drugs clinically useful, but not yet scientifically proven. Hence, the mixture of *Harithaki* (*Terminalia chebula* Retz.) and *Bhadra* (*Aerva lanata* Juss.) mentioned in *Yogamritham Pandurogachikitsa adhyayam*^[3] which provides a scientific validation to its haematinic activity can definitely become beneficial to the society as it makes less chance of adulteration, less expensive and easy home remedy for anaemia. This study was done to analyse the effectiveness of this combination using quasi experimental trial and is discussed here.

Materials And Methods:

Inclusion criteria

- Male participants having the range of Haemoglobin level 8-12.9 g/dl and female participants having the range of Haemoglobin level 8-11.9 g/dl.
- Participants with microcytic hypochromic anaemia from peripheral smear.
- Participants with RDW-CV greater than 14.

- Participants who are willing to give consent
- Exclusion Criteria
- Known cases of Anaemia due to other illnesses like peptic ulcer disease (ulcers in the stomach or small intestine), chronic kidney disease, cancer (especially gastrointestinal cancers), iron deficiency due to blood loss from the intestine or other sites.
- Pregnant, intend to become pregnant, breastfeeding, within 2 weeks postpartum, having a positive serum or urine pregnancy test.
- Participant currently receiving iron supplementation.

Intervention:

Harithakiyogam comprising of *Harithaki* (*Terminalia chebula* Retz.) and *Bhadra* (*Aerva lanata* Juss.) are the study formulation which were dispensed as tablet. The enrolled participants were given Albendazole 400-mg single dose orally for deworming before dispensing the study tablet. Then they were advised to take 1 tablet weighing 500 mg thrice daily after food. The trial Ayurveda intervention is manufactured from College Pharmacy and the punching of tablets were done from GMP Certified Pharmaceuticals.

Preparation and Standardisation of Intervention:

The raw drugs were carefully cleaned, shade dried and kept in airtight containers. Equal parts of the two drugs (1:1 ratio) were separately powdered and made into coarse powder. Similarly, equal number of drugs were powdered and made it fine after passing through the mesh of nominal aperture size 180 micrometre and kept separately in the same type container. Then these fine powders of both drugs mixed together and prepared *Harithakiyogachurna*. Then the coarse powder of *Harithakiyogam* was taken and 8 times of water was added and reduced to ⅛ as per the method

mentioned in the classical Ayurvedic literature *Bhaishajya ratnavali*.^[4] The fine powder of *Harithakiyogam* was subjected to grinding in the *kashaya* obtained till the complete absorption of the liquid into the powder and the paste obtained was subjected to drying. It takes about 6-7 hours to become soft and fine. Same process repeated 2 times for the potentiation of the *churna* and for dose reduction. The resultant grinded, potentiated and dried powder of *Harithakiyogam churnam* was made into

tablets each weighing 500mg after adding 0.2% preservative and 5% binders.

Standardisation of *Harithakiyogam* Tablet:

Individual drugs procured and ensured to meet the authentic standardisation parameters after pharmacognostical analysis, physicochemical and phytochemical analysis, ICP-MS Analysis, TLC and HPTLC Analysis. All the parameters were on a par with the API limit for individual drugs. Standardisation parameters of tablet shown in Table 1.

Table 1. Standardisation of *Harithakiyogam* Tablet:

Sr.No.	Parameters	<i>Harithakiyogam</i>
1	Foreign matter	Nil
2	Moisture content	5.39%
3	Total ash	7.16%
4	Acid insoluble ash	0.51%
5	Water insoluble ash	3.17%
6	Alcohol soluble extractive	28.74%
7	Water soluble extractive	45.8%
8	Total sugar	15.5%
9	Reducing Sugar	11.25%
10	pH	3.89
11	Disintegration time	1.22 hr
12	Friability test	0.1%
13	Hardness test	16 kg/cm ²

From the data, it showed that major drawback of the preparation of medicine after the procedure is the highest disintegration time and hardness. It makes the tablet a long time to dissolve in water.

Administration of tablet to the participants is advised after powdering it and mixed with lukewarm water.

HPTLC Analysis: HPTLC analysis of *Harithakiyogam* and its individual

component drugs showed best separation in Toluene: Ethyl acetate: Chloroform: Formic acid (4:8:1:3) shown in Figure 1

Track 1 - Gallic acid (Marker compound)

Track 2 - *Terminalia chebula* Retz. (2.5 µL) (Raw Drug)

Track 3 - Harithakiyogam (2.5 µL) (Formulation)

Track 4 - *Aerva lanata* Juss. (2.5 µL) (Raw Drug)

From the data obtained after the graphical representation (Figure 2) of 4 tracks at 254 nm, there are common Rf values shared by the Gallic acid standard and other components which is 0.979, 0.981, 0.982 and 0.984 for Gallic acid, *Terminalia chebula* Retz., *Harithakiyogam*, *Aerva lanata* Juss. respectively. While comparing the graphical representation of 4 tracks at 366 nm (Figure

3), common Rf value obtained were 0.982 for Gallic acid and *Terminalia chebula* Retz., 0.985 for *Harithakiyogam* and 0.987 for *Aerva lanata* Juss. From this, we can analyse that almost Rf values of all the single drugs and combination is similar to that of reference standard gallic acid and can infer the presence of gallic acid standard in the individual drugs and the combination.

Outcome measures:

The primary outcome measure is the change in hemoglobin level which was assessed at baseline and at 90th day. The secondary outcome measures are changes in red blood cell count, red cell indices, Peripheral smear and RDW-CV were assessed at baseline and 90th day. Secondary outcome of the study is improvement in Subjective parameters [Table 2] at baseline and 90th day.

Table 2. Subjective Parameters^{[5] [6] [7]}

Parameters	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Pandu Varna (Pallor)	No pallor	Pallor of conjunctiva or mucous membrane (Mild)	Pallor of conjunctiva, mucous membrane & skin (Moderate)	Pallor of conjunctiva, mucous membrane, skin, palmar creases (Severe)	
Shwasa (dyspnoea)	No breathlessness	Breathless when hurrying or walking up a hill (Mild)	Breathless when walking slower than people of same age or has to stop when walking (Moderate)	Stops for Breath after walking about ~100 m or after a few minutes on level ground (Moderate)	Breathless when dressing or not able to leave the house (Severe)
Reduced appetite	Normal instinct to have food	Loss of appetite without	Oral intake decreased without significant weight	Inadequate oral caloric or fluid	

		alteration in eating habits (Mild)	loss, dehydration or malnutrition (Moderate)	intake; tube feeding, TPN, or hospitalizati on indicated (Severe)	
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Fatigue was assessed based on Fatigue Assessment Scale^[8]

Participant Timeline

A total of 35 participants were screened and 5 participants were excluded as they failed to meet the inclusion criteria. 30 participants were met with inclusion criteria but 3 of them refused to take part. However, 27 participants were included but 4 participants were withdrawn from the study. Consequently, 23 participants were considered for statistical analysis. [Figure 4]

Sample Size

Sample size calculated was 25 from Previous study.^[9] By considering mean difference in Hb as 1.19, and average standard deviation of Hb from previous study is 1.455 with 90% power and considering an expected dropout rate of 10%, a total of 25 participants was calculated as sample size in the study.

Statistical Analysis

To find out the impact of the intervention, paired t-tests were performed to compare pre-treatment and post-treatment measurements for each outcome. A significance level of $P < 0.01$ was established to determine statistical significance.

Results:
Demographic Profile of the Study
Participants:

Total 23 participants completed the trial. Among which, 78.3% were females which proves the prevalence of iron deficiency anaemia in women. 47.8% participants were in age group 40-50. This may be due to the unsatisfactory and irregular food habits in both sexes whereas in females, there may be a chance of premenopausal symptoms also where heavy bleeding persists which were prevalent in this age group. Among the domicile distribution, 82.6% participants were belonged to rural area and 78.3% were from middle class in which there may be an inadequate intake of dietary iron supplements and fruits which enhances iron absorption leading that IDA more specifically caused due to nutrient deficiency. While analysing the occupation, 39.1% are of student category and 39.12% are working in public and private sector. Due to the work habit or nature of work, there might be a chance of irregular food intake or skipping of 1 or 2 meals per day which may lead to IDA. Demographic profile depicted in Table 3.

Table 3. Demographic Profile of the Study Participants:

Variables	%	Variables	%
Gender		Occupation	
Male	5(21.7%)	Student	9(39.1%)
Female	18(78.3%)	Public	6(26.08%)
Age in years		Private	3(13.04%)
20-30	9(39.1%)	Unemployed	5(21.73%)
30-40	3(13.04%)	Diet	
40-50	11(47.8%)	Vegetarian	5(21.7%)
Domicile		Mixed	18(78.3%)
Urban	4(17.4%)		
Rural	19(82.6%)		
Economic status			
Poor	5(21.7%)		
Middle	18(78.3%)		

Effect of Treatment in trial group:

In trial group, the mean Hb increased from 9.51 to 11.54 with an increase of 2.03(g/dl) after the treatment. The mean RBC increased from 3.96 to 4.36 with an increase of 0.40(million/mm³) after the treatment. The mean MCV increased from 70.24 to 81.74 with an increase of 11.5(fl) after the treatment. The mean MCH increased from 22.65 to 27.93

with an increase of 5.28(pg/cell) after the treatment. The mean MCHC increased from 30.58 to 33.98 with an increase of 3.4(g/dl) after the treatment. The mean RDW-CV decreased from 16.7 to 13.55 with a decrease of 3.14(%) after the treatment. All the parameters were significant at 1% level of significance (p-value<.01) (Table 4). Graphically represented in Figure 5.

Table 4. Effect of Treatment in trial group:

Parameters	Mean ± SD BT	Mean ± SD AT	Difference in mean	t-value	p-value
Haemoglobin	9.51±0.95	11.54±0.47	-2.03	-11.17	< 0.01
RBC	3.96±0.38	4.36±0.28	-0.4	-7.78	< 0.01
MCV	70.24±4.14	81.74±4.17	-11.5	-13.66	< 0.01
MCH	22.65±1.86	27.93±2.21	-5.28	-14.29	<0.01
MCHC	30.58±1.35	33.98±0.87	-3.4	-11.15	< 0.01
RDW-CV	16.7±1.14	13.55±0.81	3.14	12.75	< 0.01

Effect of Treatment in Peripheral Smear:

Before treatment all cases were microcytic hypochromic, 73.9% became normal after the treatment and only one remains microcytic hypochromic. (Table 5) (Figure 6)

Table 5. Effect of Treatment in Peripheral Smear:

Peripheral Smear BT (n - 23)	Peripheral Smear AT				Total
Microcytic Hypochromic	Normal	Normocytic normochromic	Dimorphic (Normocytic normochromic to microcytic hypochromic)	Microcytic hypochromic	
23	17	1	4	1	23
100%	73.9%	4.3%	17.4%	4.3%	100.0%

Effect of treatment in Subjective Symptoms:

Clinical improvement in subjective parameters was shown in Table 6.

Table 6. Effect of treatment in Subjective Symptoms:

Pallor	BT	AT
Normal	Nil	78.26%
Mild	43.47%	21.73%
Moderate	56.52%	Nil
Reduced Appetite	BT	AT
Normal	26.08%	78.26%
Mild	43.47%	21.74%
Moderate	30.43%	Nil
Shortness of breath	BT	AT
Normal	Nil	73.26%
Very mild	17.39%	17.39%
Mild	39.13%	8.69%
Moderate	43.47%	Nil
Fatigue	BT	AT
Normal	Nil	86.95%
Mild to Moderate	100%	13.04%

DISCUSSION:

Iron deficiency anaemia has variable causes such as impaired production of red blood cells, excessive destruction of cells, inadequate nutritional iron, excess blood loss, parasite infection leading to the non-absorption of iron etc. *T. chebula* ethanol extract acts as a natural anthelmintic that has produced inhibitory effects on the action of Acetylcholinesterase (AChE) by blocking AChE activity and also decreases the motor activity of *Cotylophoron cotylophorum* in a time-dependent concentration manner. Halting the motility of worms and a repercussion from the intestinal peristalsis of the host also occurred.^[10] Also, Aqueous and Alcoholic extracts obtained from the leaf and stem of plant *Aerva lanata* Juss. possess good anthelmintic activity as compared to albendazole.^[11] This anthelmintic effect of both drugs is able to prevent the further formation of worms in intestine and thereby increasing iron absorption.

Hepcidin is an Iron regulator hormone in which the release of hepcidin increases during inflammatory condition. It can able to bind with ferroportion and cause degradation which results in anaemia. Total and Reducing Sugar present in the drugs have anti-inflammatory action thereby reducing the excess release of hepcidin and in turn correcting the metabolism and may cure anaemia.^[12]^[13]

While coming to the ayurvedic treatment, diagnosis of IDA with respect to Ayurveda is important. *Pandu* (pallor/clinical features of pāṇḍu) altogether or its specific types simply cannot be directly correlated to Iron Deficiency Anaemia (IDA) because the concising of this broad term not easily possible. Although, while going through the *samprapthi*(pathogenesis) of *panduroga*(pallor/clinical features of pāṇḍu) and pathogenesis of IDA, *rakthakshaya*(depletion of blood) or reduction in haemoglobin is a common

factor and while comparing the symptoms of both, reduced appetite, dryness of skin, fatigue, weakness, dyspnoea on exertion, palpitation & pallor of the skin, mucous membranes & sclera, tinnitus, unusual dietary cravings such as pica^[14] can be correlated with *Agnimandya*(diminution of agni), *Twakrukshatha*, *Angamarda*, *Dourbalya*, *Srama*(exhaustion/fatigue), *hridayaspandana*, *Panduvarnata*(Pallor), *Karnanada*(ringing in the ears/ tinnitus), *Mritbhakshanaecha* etc. respectively. Hence, considering all these, IDA can be considered in the spectrum of *Panduroga*(pallor/clinical features of pāṇḍu). *Panduroga*(pallor/clinical features of pāṇḍu) is a *pittapradhana tridoshaja vyadhi* in which *Tridoshas*(three regulatory functional factors of the body), *Rasa*(primary product of digested food), *Raktha*(blood tissue), *Medodhathus* and *Rasavaha*(channels carrying nutrient fluids) - *Rakthavaha srothas*(channels carrying blood tissue) are involved in the *samprapthi*(pathogenesis) and has affecting *jataragni*(metabolic factors located in digestive tract) and *rasa-rakthadhatwagni*(metabolic factors located in dhātu) also. Due to *ahara - viharaja nidana*(aetiology) of *Panduroga*(pallor/clinical features of pāṇḍu) and *vishamasana*, *adhyasana* causing *jataragnimandyathwam*(diminution of metabolic factors located in digestive tract) and thereby vitiating *rasa raktha dhathus*(primary product of digested food and blood tissue) and formation of *ama* which got *sthanasamsraya*(stage of localization) in the *srotasas*(structural or functional channels) and causing *srothorodha*(obstructive pathology occurring in channels) which ultimately cause impairment in the *dhathupaka*. Here, a quantitative increase of *pittadosa*(doṣa responsible for regulating body temperature and metabolic activities) with which it reduces the *tikshna*(sharpness)

usna(hotness) *swabhava* and increases *drava*(fluidity) *sara*(instability/mobility) *guna* of *pittadosha*(*doṣa* responsible for regulating body temperature and metabolic activities) which causes *kaphavilayanatwa* thereby *rasadushti* turns to the inappropriate *dhathu*(major structural components of the body) transformation and reduction in quantitative and qualitative formation of *rakthadhathu*(blood tissue). Due to the loss of specific *gunas* of *pitta* (*doṣa* responsible for regulating body temperature and metabolic activities), it will be equivalent to *kapha* (*doṣa* responsible for regulating body fluids and keeping the body constituents cohesive) and *vikrtavarna* or *pandutwa*(Pallor) occurs. And there is qualitywise diminished *pittavardhana* (*Swagunahanipitta*) is happening and results in *rakthakshaya*(depletion of blood tissue). The term *Rakthakshaya* indicates the reduction in viscosity and increase in the fluidity or *sidhilatha* of *rakthadhathu*(blood tissue). The *rakthakshaya*(depletion of blood tissue) causing *vatakopa*. As the *rasavahasrothomoola* is *hridaya*, the *rasadushti* causes *vyanavayudushti* (vitiation of a subtype of *vāta*, that is seated in *hṛdaya*) also and results in *pratilomagati* of *vyanavayu*(a subtype of *vāta*, that is seated in *hṛdaya*) and circulates throughout the body which gives rise to loss of integrity of that *dhathu* (*sidhilatha*), *Gourava* (heaviness of the body), *vaivarnya* (abnormal change of complexion), *bala kshaya* (loss of strength), *sneha kshaya* (loss of unctuousness/depletion of normal fat) and ultimately *ojokshaya*(depletion of essence of all seven *dhātu*). Thus, the drug of choice should have *apakwapitta sodhana*, *agnideepana*(increase in power of *agni*), *pachana*(enhancing digestion), *srotosodhana*(cleanliness of *srotas*/ felling of cleanliness of *srotas*), *rasayana*(rejuvenation) properties.

Harithaki (*Terminalia chebula* Retz.) is one of the best *anulomaka*(mild purgative action/regularizing physiological movement) *Dravya* (material or substance used for therapeutic purpose or health benefits). *Harithaki* with its *Deepana* (digestion and metabolism enhancing), *anulomana karma* can capable of normalise the *agni* and *pratilomagati* of *vyanavayu* and results in *anulomana* of *vayu* and *purisha* also. Also, it is having the karma of *Yakrtuttejaka*, hence potential to regularize *rakthavahasrothomoola* which ultimately improve the process of *raktha* formation.^[15] *Bhadra* is having *tiktha*, *kashaya rasa* can contradict the *gunas* (attribute/ property) of *pitta*(*doṣa* responsible for regulating body temperature and metabolic activities) and *Tikshna*(sharpness) ,*usna*(hotness) *guna*(attribute/ property) of *Bhadra* act as *srothosodhana*(cleanliness of *srotas*/ felling of cleanliness of *srotas*) and improving the *jatharagni*(metabolic factors located in digestive tract) thereby evacuating the *apakwapitta*. Also, plant possess less toxicity towards erythrocyte membrane which has been proved by in-vitro research and also have anti-microbial, anti-parasitic, anthelmintic activity.^[16] The study participants were having irregular food habits which paves to the formation of *ama*. Correction of *jatharagni*(metabolic factors located in digestive tract) is very important in the proper formation of *pitta* (*doṣa* responsible for regulating body temperature and metabolic activities). *Agnimandya* (diminution of metabolic factors located in digestive tract) which is the ultimate cause of *Pandu* (Pallor) which leads to *amatva*, *srotorodha*(obstructive pathology occurring in channels) and improper *rasadhatu* formation. *Usnavirya*(hot potency), *Deepana* (increase in power of *agni*), *Pachana*(enhancing digestion) properties of the study drugs will correct the *jatharagni*(metabolic factors located in

digestive tract). Also, both the drugs having *kashaya rasa* which exhibit the properties like *asravisodhana* and *atitwakprasadana*. Thus, the quality of *raktadhatu*(blood tissue) could be improved.

CONCLUSION:

The Study drug *Harithakiyogam* tablet (Combination of *Harithaki* & *Bhadra*) 500 mg thrice daily after food for 3 months in 23 study participants is effective in improving Haemoglobin count in Iron Deficiency Anaemia and was statistically significant also. The tablet is effective in improving red cell indices parameter of study participants. Peripheral Smear of 73.9% of study participants became normal after the trial. The Study drug also shows improvement in the clinical symptoms of IDA like Pallor, loss of appetite, fatigue, shortness of breath etc. which are the most affected symptoms of study participants. The Study drug is also found to be a safe combination in management of IDA as no adverse effects were observed during the study.

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Figure Legends

1. Individual components and the formulation showed best separation in the solvent

2. Rf Values shared by the tracks were almost similar which indicates the presence of Marker Gallic acid in components and the formulation
3. Under UV Long (366 nm), similar Rf values were obtained which indicates the presence of Marker Gallic acid in components and the formulation
4. Consort Diagram of Study Participants
5. All the outcome parameters showed significant effect after the trial
6. Peripheral smear of study participants showed statistical significance after the treatment.

CTRI Registration Date - 09/11/2023

List of Abbreviations

IDA - Iron Deficiency Anaemia

HPTLC - High Performance Thin Layer Chromatography

Rf Value - Retention Factor

RDW-CV - Red Cell Distribution Width - Coefficient of Variation

Source of Support - Nil

Conflicts of interest - Nil

Authors Contribution: All authors have contributed equally.

Financial Support and Sponsorship: None declared

Conflict of Interest: There are no conflicts of interest.

Declaration of Generative AI and AI Assisted Technologies in the writing process:

The author has not used generative AI/AI assisted technologies in the writing process.

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Website: <https://www.ijim.co.in> **Email:** ijimjournal1@gmail.com

IIFS Impact Factor: 4.125

Frequency of Publication: Monthly