

# Efficacy of Fortified Finger Millet Cookies with and Without *Trikatrayadi Lauha* versus Pearl Millet Cookies for Iron Deficiency Anaemia in Preschool Children: A Randomised Controlled Trial Research Protocol

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## ABSTRACT

**Introduction:** Iron Deficiency Anaemia (IDA) is widely prevalent among preschool children in India and poses a major health concern. Ayurveda describes Pandu as analogous to IDA, emphasising the importance of nutritional interventions.

**Need of the study:** Millets have been selected as the primary food-based intervention due to their beneficial properties, including *Tikta*, *Madhura* and *Kashaya rasa* (bitter, sweet and astringent tastes), *Shita virya* (cold potency), *Snigdha guna* (unctuousness) and *Balya* (strength-promoting qualities). Pandu is considered a *Pitta*-dominant disorder and millets are known to help in pacifying *Pitta*. Furthermore, the *Snigdha* and *Balya* properties may help reduce dryness, tissue weakness, emaciation and degeneration as described in the pathogenesis of Pandu. Cookies have been chosen as a convenient delivery form to improve acceptability and compliance. The incorporation of *Pippali*, *Anjir* and *Kismish* (PAK), along with *Trikatrayadi Lauha Kalpa*, is intended to enhance the nutritional and therapeutic value of the formulation.

Thus, there is a need to explore effective food-based interventions for the management of Pandu and this study aims to contribute to this area.

**Aim:** To compare the efficacy of PAK (*Pippali*, *Anjir*, *Kismish*)-fortified finger millet cookies with and without *Trikatrayadi Lauha* and PAK-fortified pearl millet cookies in children with IDA.

**Materials and Methods:** A triple-arm, randomised, double-blind, superiority-controlled clinical trial will be conducted at Mahatma Gandhi Ayurved College Hospital and Research Centre (MGACH and RC), Sawangi (Wardha), Maharashtra, India, along with nearby Government Anganwadi centres and Government Vaccination Centres in Wardha district from March 2026 to September 2026. A total of 180 children aged 3-6 years diagnosed with mild to moderate anaemia will be enrolled. Participants will be randomised into three equal groups using computer-generated block randomisation. Group-X: Finger millet cookies fortified with *Trikatrayadi Lauha*, Group-Y: Finger millet cookies only, Group-Z: Pearl millet cookies. Each child will receive two cookies daily for 90 days. Haemoglobin levels and clinical parameters will be assessed at baseline and at the end of the intervention period. Continuous variables will be expressed as Mean±Standard Deviation (SD) and compared using Analysis of Variance (ANOVA). Categorical variables will be analysed using the Chi-square test. A p-value <0.05 will be considered statistically significant.

**Keywords:** Ayurveda medicine, Child, Haemoglobin, Nutritional intervention

## INTRODUCTION

The IDA is the most prevalent nutritional deficiency worldwide, with a particularly high burden in India, where 67% of children under five are affected according to the National Family Health Survey (NFHS-5, 2019-2021) [1]. IDA impairs cognitive development, immunity and growth in children, leading to long-term health and developmental consequences [2]. The World Health Organisation (WHO) defines anaemia in preschool children as Haemoglobin (Hb) <11.0 g/dL [3]. Despite the availability of free iron supplementation programs, the prevalence of anaemia remains high due to side-effects, poor compliance and limited dietary diversity [4].

Three primary strategies are employed worldwide to manage IDA: supplementation with iron and folic acid tablets, food fortification and natural food-based approaches. Despite the widespread use of the first two methods, IDA remains a major cause of malnutrition. The third approach—dietary diversification with naturally iron-rich foods—avoids the side-effects of artificial additives [5].

Millets, especially finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*), are rich in iron, minerals, fibre and vitamins and have demonstrated efficacy in improving haemoglobin levels

in children. Finger millet (*ragi*) is a nutrient-dense small millet with a favourable micronutrient profile: whole-grain *ragi* contains appreciable amounts of iron and calcium, high dietary fibre, a balanced amino-acid profile relative to other cereals and several phenolic antioxidants that may confer additional health benefits [6,7]. Pearl millet is notable for its high iron and zinc concentrations, agronomic resilience in drought-prone regions, making it an attractive staple for food-based approaches to micronutrient deficiencies [8,9].

*Trikatrayadi Lauha Kalpa* is used in this study as a Herbo-Mineral formulation containing *Triphala*, *Trikatu* and *Trimada* [10]. *Triphala* acts as a *Rasayan*, *Trikatu* as an appetiser and *Trimada* acts as *Pachan*. Particularly, *Pippali* is added as it is an excellent *Rasayan*, as said by *Bhavpraksash* [11]. Because of its *Rasayana* property, it strengthens each *Dhatu*, resulting in enhanced immunity. It is easily available in the market in pure form and palatable in particular doses. *Anjir* has properties like *Tarpak* (Nourishing), *Bruhan* (Nutritious) and *Sheet Virya*, which are very useful to alleviate *Pitta*. *Tarpak* and *Bruhan* reduce the *Dhatu*. According to Ashtang Hridaya [12], *Kismish* (Black Raisin) has *Madhur* and *Kashay Rasa*, *Sheet Virya*, which alleviates *Vat pitta* and *Rakta pitta*. It reduces *Kshaya* (Deficiency), increases appetite and *Sara Guna* prevents constipation [13].

Ayurveda correlates IDA with Pandu Vyadhi. Imbalance in one *Dhatu* also leads to imbalance in the other. In *Pandu Vyadhi*, mainly *Ras Dhatu Dushti* takes place. If no treatment is given, it leads to dryness and *Shaythilya* of all *Dhatu* and depletion of *Ojas*. This leads to many symptoms like tinnitus, anorexia, loss of fat, weakness, fever, body pain, periorbital swelling, etc. Anaemia recommends dietary interventions using nutrient-rich foods and herbal formulations. There are numerous millet recipes, including millet cookies, *Roti*, *Khir*, *Dosa*, etc., But cookies are the most visually appealing, easily palatable, storable, convenient and quick-serving foods. Cookies, being palatable and convenient, offer an effective medium for delivering nutritional interventions to children.

This protocol outlines a randomised controlled trial to evaluate the comparative efficacy of fortified finger millet cookies with and without *Trikatrayadi Lauha* versus pearl millet cookies in improving haematological parameters among preschool children with IDA.

**Aim:** To evaluate the comparative efficacy of PAK-fortified finger millet cookies with and without *Trikatrayadi Lauha* and pearl millet cookies in the management of *Pandu* (IDA) in preschool children.

**Primary objectives:** To study the efficacy of PAK-fortified finger millet cookies with *Trikatrayadi Lauha* in the management of *Pandu* (IDA) in preschool children;

To study the efficacy of PAK-fortified finger millet cookies in the management of *Pandu* (IDA) in preschool children;

To study the efficacy of PAK-fortified pearl millet cookies in the management of *Pandu* (IDA) in preschool children.

**Secondary objectives:** To compare the efficacy of PAK (*Pippali*, *Anjir*, *Kismish*)-fortified finger millet cookies with and without *Trikatrayadi Lauha* and PAK-fortified pearl millet cookies in the management of *Pandu* (IDA) in preschool children.

## Hypothesis

**Null hypothesis ( $H_0$ ):** The PAK-fortified finger millet cookies with and without *Trikatrayadi Lauha* are not superior to PAK-fortified pearl millet cookies in the management of *Pandu* (IDA) in preschool children.

**Alternative hypothesis ( $H_1$ ):** PAK-fortified finger millet cookies with and without *Trikatrayadi Lauha* are superior to PAK-fortified pearl millet cookies in the management of *Pandu* (IDA) in preschool children.

## REVIEW OF LITERATURE

The IDA is a leading cause of morbidity in children globally, with the highest prevalence in low- and middle-income countries. The aetiology of IDA includes inadequate dietary intake, poor absorption and increased requirements during growth [14]. Early epidemiological studies highlighted that inadequate dietary iron intake, poor bioavailability, recurrent infections and increased physiological demands during periods of rapid growth are primary contributors to paediatric IDA [15]. Eight research studies were carried out, which include human interventions and in-vitro studies (considering studies published between 2010 to 2020). The studies covered a range of outcomes, including Serum Ferritin and Haemoglobin levels. Finger millet, pearl millet, teff, sorghum, or a combination of millets were all used in the studies.

The findings of 19 investigations on anaemic people demonstrated a noteworthy rise in haemoglobin levels by 13.2% after regularly consuming millet compared with a regular diet, where there was only 2.7% increase [5].

Anitha S et al., investigated whether millet consumption could reduce IDA. The findings indicated that millets are an excellent and cost-effective source of dietary iron, with strong potential for reducing the prevalence of IDA [5].

Kodkany BS et al., conducted a study to determine the absorption of iron and zinc from pearl millet biofortified with two micronutrients

that are commonly deficient in non fortified, plant-based diets globally. The results demonstrated that the enhanced concentrations of iron and zinc achieved through biofortification- primarily targeting increased iron content- were bioavailable and sufficient to meet physiological requirements [16].

Scott SP et al., evaluated the efficacy of iron-biofortified pearl millet compared with conventional pearl millet in improving attention and memory among Indian school-going adolescents. The study concluded that consumption of iron-biofortified pearl millet led to better outcomes than conventional pearl millet, with significant improvements in iron status and certain measures of cognitive performance [17].

Devulapalli D and Gokhale D, assessed the nutritional impact of millet-based foods, including millet health mix, energy bars, finger millet cutlets and biscuits, on body weight, Body Mass Index (BMI) and haemoglobin levels before and after intervention. The results supported the study hypothesis, demonstrating that millet-based foods significantly improved weight, BMI and haemoglobin levels among adolescent girls [18].

In previous work, all types of millet or a mixture of millets were given to individuals. In this study, the authors are comparing the efficacy of *Pippali*, *Anjir* and *Kismish* fortified Finger millet cookies with and without *trikatrayadi lauha* and Pearl millet Cookies, which is better for increasing Haemoglobin status in preschool children.

All the above articles have a knowledge gap as they create confusion among researchers and practitioners about which specific millet should be used for IDA in children.

## MATERIALS AND METHODS

A randomised, triple-arm, double-blind, superiority-controlled trial will be conducted among 180 preschool children (3-6 years) with mild anaemia (Hb 9-11 g/dL) at MGACH and RC Hospital, Wardha, Maharashtra, India, from March 2026 to September 2026. The study received approval from the Institutional Ethics Committee of Mahatma Gandhi Ayurved College, Hospital and Research Centre (IEC No: MGACHRC/IEC/March-2025/915) and with registration (CTRI/2025/05/086399). Written informed consent and assent were obtained from all participants before enrollment. Confidentiality of participant data was maintained according to national ethical and regulatory guidelines.

**Inclusion criteria:** A total of 180 preschool children (boys and girls) aged 3-6 years with Hb <11 g/dL after obtaining a written informed consent from parents/guardians.

**Exclusion criteria:** Participants with chronic illnesses will be excluded (e.g., sickle cell anaemia, thalassemia, tuberculosis, worm infestation, bronchitis, sinusitis), active infectious disease, haemoglobin <9 g/dL, or prior hypersensitivity to trial product components.

**Sample size calculation: It was calculated using the formula:**  

$$n = \frac{2(Z\alpha + Z\beta)^2 \sigma^2}{\Delta^2}$$

The pre-post-test improvement in proportion of iron deficiency after the proposed intervention (i.e., Fortified Finger Millet) among pre-school children with IDA as the main outcome measure for the effect size.

Pre-test (at baseline) proportion with IDA: 53.2% [19].

Post-test (after 3 months of intervention) proportion with IDA: 28.2%, based on the assumption that 20-25% of children will become free from iron deficiency anaemia after treatment.

Effect size (Reduction in IDA)= 20-25%.

Difference in proportions: 25.0%

Power (1 - Beta): 80%

Alpha error (2-sided): 0.05

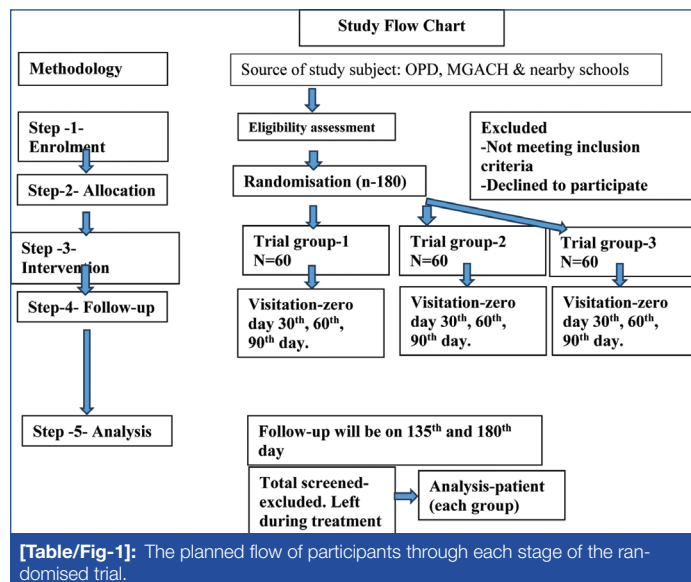
Required sample size (n): 60

The calculated sample size would be around 60 participants per group:

Since this is a three-arm trial, the total sample size will be  $n=60 \times 3=180$

Therefore, a total of 180 subjects will be included in this study.

Three independent representative samples of 60 subjects each who fulfil all the eligibility (inclusion and exclusion) criteria, consecutive by the stratified sampling method from different sections of the pre-school, as per the following plan [Table/Fig-1].



## Study Procedure

All subjects and the investigator will be blinded to treatment assignment. Participants will be randomly allocated to either the intervention or control group. Both groups will receive identical cookies wrapped in butter paper and packaged in brown paper bags, labelled with coded identifiers prepared by an independent third party. Allocation concealment will be done by the Sequentially Numbered, Opaque Sealed Envelopes (SNOSE) Method. Use of block randomisation and unpredictable computer-generated random sequences will help mask or conceal the treatment assignment to subjects.

**Cookie preparation:** Raw materials required for the preparation of millet cookies and *Trikatrayadi Lauha* will be procured from authenticated and Good Manufacturing Practice (GMP)-certified suppliers. Finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) grains will be obtained from certified local agricultural suppliers and will be cleaned and processed under hygienic conditions.

Herbal ingredients used for the preparation of *Trikatrayadi Lauha*, including *Triphala* (*Haritaki*, *Bibhitaki*, *Amalaki*), *Trikatu* (*Shunthi*, *Maricha*, *Pippali*) and *Trimada*, will be procured in crude form from a GMP-certified Ayurvedic pharmacy. All herbal drugs will be authenticated by a *Dravyaguna* expert, with verification based on classical Ayurvedic texts and the standards outlined in the Ayurvedic Pharmacopoeia of India. The cookies will be standardised and prepared under GMP-certified conditions using a uniform formulation (per 14 cookies) comprising finger/pearl millet flour (119 g), wheat flour (119 g), jaggery powder (119 g), *Pippali churna* (17.5 g), Anjir (22 g), raisins (22 g), ghee (62 g) and cardamom (1 g).

All the ingredients will be carefully measured and thoroughly mixed in a clean bowl until a uniform dough is formed. The prepared dough will be kept in the refrigerator for a specified period to firm up and enhance its texture. After refrigeration, the dough will be removed and shaped into uniform portions or desired cookie forms. These

shaped pieces will then be arranged on a baking tray and baked in a preheated oven at 180°C for approximately 12-14 minutes.

Participants will receive daily nutritional supplementation, fortified cookies for 90 days.

Participants will be instructed to take a daily dose of two cookies (34.39 gm/cookie) for 90 days with *Anupan* water.

*Trikatrayadi lauha* churna 3 gm/day will be given to the allotted group [20].

## Outcome Measures

**Primary outcome:** Change in haemoglobin after three months.

**Secondary outcomes:** Change in Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), Packed Cell Volume (PCV), serum ferritin and serum iron. All these parameters will be assessed at baseline 0 day, 30 days, 60 days, 90 days, 135 days, 180 days, as well as on Ayurveda subjective parameters such as *Daurbalya*, tolerability and adverse events.

## STATISTICAL ANALYSIS

Data will be analysed using Stata version 18 (StataCorp, Texas, USA). Descriptive statistics will be presented as mean±standard deviation for quantitative variables and frequencies/percentages for qualitative variables. Effect measures will be expressed as means or percentages with 95% confidence intervals. Between-group comparisons will be performed using an independent t-test (quantitative) and Chi-square test (qualitative). Within-group comparisons over time will be assessed using One-way ANOVA with a Bonferroni post-hoc test, while Two-way ANOVA will evaluate the effects of age and gender. If normality assumptions are violated, appropriate non parametric tests will be applied. A p-value <0.05 will be considered statistically significant.

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