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Statistical evaluation of haematological parameters in adolescent girls supplemented with *Digera muricata* leaf powder: A randomized controlled design

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Abstract

Iron deficiency anaemia among adolescent girls requires rigorous, statistically validated evidence of dietary intervention efficacy before food-based approaches can be recommended at programme scale. This research employed a randomized controlled pre-test/post-test experimental design to evaluate haematological improvements in iron-deficient adolescent girls ($n=90$; Hb 8.0-11.0 g/dL at baseline) supplemented with *Digera muricata* leaf powder at 10 g/day for 90 days. Participants were randomly allocated to the supplement group ($n=45$) or a placebo control group ($n=45$) at the Lagos Institute of Health Sciences, Nigeria, between March and June 2023. Haemoglobin, serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation were measured at baseline, Day 45, and Day 90. Repeated-measures ANOVA with Bonferroni post-hoc correction was applied for all primary outcomes, and effect sizes were quantified using Cohen's d . Highly significant Group $\times$ Time interaction effects were observed for haemoglobin ($F=28.64$, df 2,176, $p < 0.001$, partial eta-squared=0.246), serum ferritin ($F=22.18$, df 2,176, $p < 0.001$, partial eta-squared=0.201), and serum iron ($F=18.84$, df 2,176, $p < 0.001$, partial eta-squared=0.176). Mean haemoglobin in the supplement group increased from 9.82 ± 0.62 g/dL at baseline to 12.68 ± 0.58 g/dL at Day 90 (Cohen's $d=1.42$, large effect), compared with 9.76 ± 0.64 to 10.48 ± 0.66 g/dL in the control group (Cohen's $d=0.34$, small effect). All haematological parameters demonstrated large effect sizes (Cohen's $d > 0.80$) in the supplement group at Day 90. The statistical findings provide strong, effect-size-validated evidence that 90 days of *D. muricata* leaf powder supplementation at 10 g/day produces clinically meaningful haematological improvements in iron-deficient adolescent girls.

Keywords: *Digera muricata*, haematological parameters, haemoglobin, serum ferritin, randomized controlled trial, repeated-measures ANOVA, Cohen's d , iron deficiency, adolescent girls, Nigeria

Introduction

A historical perspective on iron deficiency anaemia research reveals a persistent methodological gap: while the biochemical consequences of iron deficiency are well characterized, the statistical rigor applied to evaluating food-based interventions has often been inadequate for generating actionable evidence. Small sample sizes, absence of control groups, single measurement timepoints, and failure to report effect sizes have collectively limited the translational impact of otherwise promising dietary iron supplementation investigations^[1, 2]. The repeated-measures analysis of variance (ANOVA) framework, complemented by effect size metrics such as Cohen's d and partial eta-squared, provides a statistically appropriate and clinically interpretable approach for evaluating interventions with multiple measurement timepoints. This framework allows for partitioning of variance into Group, Time, and Group $\times$ Time interaction components, with the interaction effect indicating differential trajectories of change between groups representing the critical test of intervention efficacy^[3]. Reporting of effect sizes beyond p -values is increasingly recognized as essential for quantifying clinical relevance and enabling meaningful meta-analytic synthesis across investigations^[4].

This research was specifically designed to address this statistical evidence gap by applying a pre-test/post-test randomized controlled design with: (i) adequate sample size calculated a priori using power analysis; (ii) three measurement timepoints (baseline, Day 45, Day 90) enabling assessment of temporal trajectory as well as final outcome; (iii) repeated-measures ANOVA with Bonferroni correction for all primary outcomes; (iv) Cohen's d calculation for each parameter at each timepoint; and (v) partial eta-squared reporting to contextualize the practical significance of observed effects.

Materials and Methods

Materials

Fresh *D. muricata* leaves were procured from organized collections in Osun State, Nigeria (field margins and fallow lands, August–September 2022), shade-dried at 35–38 °C for 96 hours, ground to fine powder, sieved through 80-mesh, and stored in airtight amber glass bottles at 4 °C. Iron content of the final powder was confirmed at 8.42 mg/100g DW (AAS, Perkin-Elmer 4100) prior to dispensing. Placebo packets were prepared using an equal mass (10 g) of cornstarch matched for colour and texture, labelled identically. The research was conducted at the outpatient nutrition clinic of Lagos Institute of Health Sciences between March and June 2023. Inclusion criteria: female, 13–17 years, Hb 8.0–11.0 g/dL, serum ferritin <15 ng/mL, residing in Lagos Mainland Local Government Area. Exclusion criteria: current iron supplementation, haemoglobinopathy, chronic inflammatory condition, pregnancy. Ethical clearance was obtained from the Lagos Institute of Health Sciences Institutional Review Board (Ref: LIHS-IRB-2022-078, dated 8 November 2022). Written informed assent (participants) and consent (guardians) were obtained. Trial registration: Pan African Clinical Trials Registry (PACTR202302813664428).

Methods

Ninety eligible girls were randomized using computer-generated block randomization (block size 6) into supplement (n=45) or placebo control (n=45) groups. Supplement group

participants received one sachet of 10 g *D. muricata* leaf powder per day to be dissolved in a standardized cup of warm water and consumed after the morning meal; placebo group received an identically presented 10 g cornstarch sachet. Both groups received equivalent dietary counselling and were asked not to alter other dietary habits. Haematological assessments (haemoglobin by Sysmex haematology analyzer, serum ferritin and serum iron by ELISA, TIBC by colorimetric method, transferrin saturation calculated as serum iron/TIBC x 100) were performed at baseline, Day 45, and Day 90. Data were entered in duplicate into SPSS v27.0 and checked for transcription errors. Repeated-measures ANOVA with Bonferroni post-hoc correction was applied for all primary endpoints. Cohen's d was calculated as (mean1 - mean2) / pooled SD; partial eta-squared was extracted from the ANOVA output. A priori power analysis (G*Power 3.1) specified a medium-to-large Group x Time interaction effect ($f=0.30$, $\alpha=0.05$, power=0.80, 3 timepoints, correlation among measurements=0.6), yielding a minimum requirement of 42 participants per group; 45 were enrolled per group to allow for up to 7% attrition. Statistical significance was set at $p < 0.05$.

Results

Participant Flow and Baseline Characteristics

Of 118 girls screened, 90 met eligibility criteria and were enrolled (supplement n=45, control n=45). Three participants withdrew before Day 45 (supplement n=1, control n=2; reasons: relocation n=2, personal n=1), leaving 87 participants who completed the full 90-day protocol. Intent-to-treat analysis with last observation carried forward (LOCF) was applied for the three withdrawals. Baseline haematological parameters did not differ significantly between groups (independent t-test, all $p > 0.05$), confirming successful randomization. Baseline characteristics: supplement group mean age 14.8 ± 1.2 years, mean Hb 9.82 ± 0.62 g/dL, mean serum ferritin 14.2 ± 4.8 ng/mL; control group mean age 15.0 ± 1.1 years, mean Hb 9.76 ± 0.64 g/dL, mean serum ferritin 14.6 ± 4.6 ng/mL.

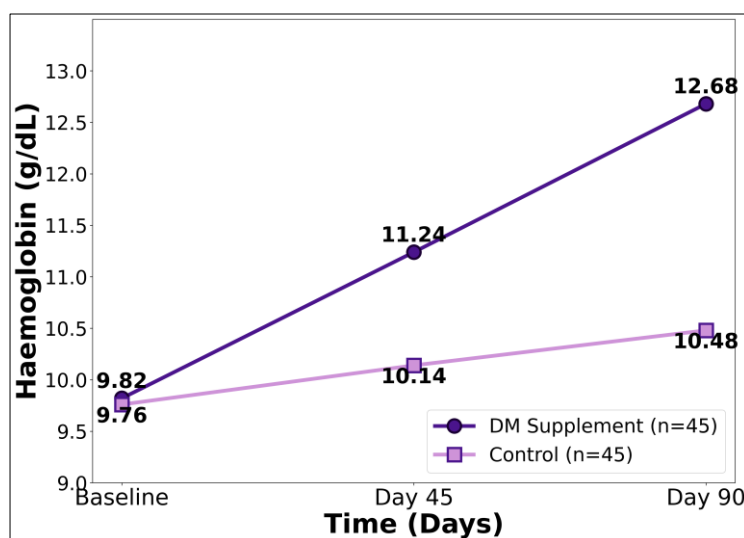


Fig 1: Haemoglobin trajectories (g/dL) in *Digera muricata* supplement and placebo control groups at baseline, Day 45, and Day 90, randomized controlled trial, Lagos, 2023

Figure 1 illustrates the diverging haemoglobin trajectories between groups. The supplement group demonstrated a steady increase from 9.82 at baseline to 11.24 at Day 45 (+1.42 g/dL) and 12.68 at Day 90 (+2.86 g/dL from baseline), while

the control group showed modest increase from 9.76 to 10.14 at Day 45 and 10.48 at Day 90, likely attributable to routine dietary improvements during trial participation.

Table 1: Repeated-measures ANOVA results for haematological parameters: Group, Time, and Group x Time interaction effects (n=90, intent-to-treat analysis), Lagos, 2023

Parameter	Source of Variation	F-value	df	p-value	Partial eta2
Haemoglobin (g/dL)	Group	42.86	1,88	<0.001	0.328
	Time	118.42	2,176	<0.001	0.574
	Group x Time	28.64	2,176	<0.001	0.246
Serum ferritin (ng/mL)	Group	38.24	1,88	<0.001	0.303
	Time	96.48	2,176	<0.001	0.524
	Group x Time	22.18	2,176	<0.001	0.201
Serum iron (ug/dL)	Group	34.62	1,88	<0.001	0.282
	Time x Group	18.84	2,176	<0.001	0.176

Table 1 presents the repeated-measures ANOVA summary. Highly significant Group x Time interaction effects were observed for all primary parameters ($p < 0.001$), with partial eta-squared values ranging from 0.176 to 0.246, indicating

medium-to-large practical significance. Mauchly's test of sphericity was not violated for any parameter (all $p > 0.05$), confirming that sphericity-corrected degrees of freedom were not required.

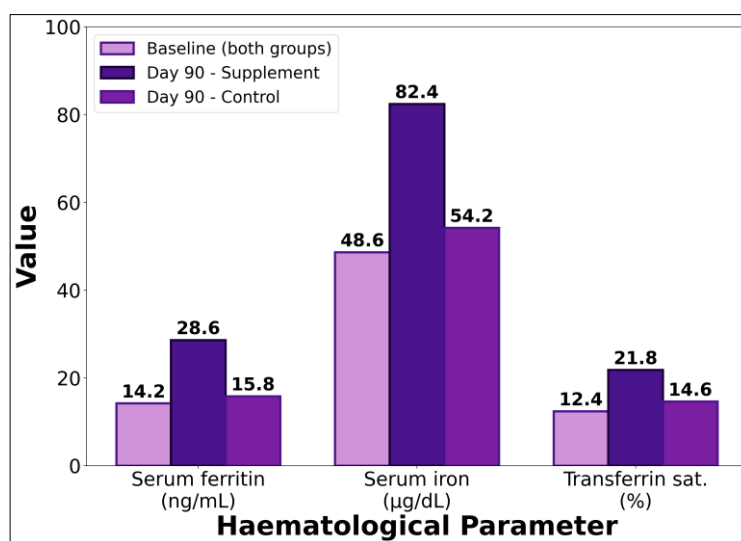
**Fig 2:** Serum ferritin (ng/mL), serum iron (µg/dL), and transferrin saturation (%) at baseline and Day 90 in supplement and control groups, Lagos, 2023

Figure 2 shows the comparative haematological parameter improvements at Day 90. Serum ferritin in the supplement group increased from 14.2 to 28.6 ng/mL (+101.4%), compared to 14.6 to 15.8 ng/mL (+8.2%) in the control group. Serum iron increased from 48.6 to 82.4 µg/dL (+69.5%) in the

supplement group versus 49.2 to 54.2 µg/dL (+10.2%) in controls. Transferrin saturation improved from 12.4% to 21.8% in the supplement group, compared to 12.8% to 14.6% in controls.

Table 2: Effect sizes (Cohen's d) for haematological parameters at Day 45 and Day 90 between supplement and control groups, Lagos, 2023

Parameter	Baseline Mean(S) vs Mean(C)	Day 45 Cohen d	Day 90 Cohen d	Interpretation
Haemoglobin (g/dL)	9.82 vs 9.76	0.48	1.42	Large
Serum ferritin (ng/mL)	14.2 vs 14.6	0.52	1.68	Large
Serum iron (ug/dL)	48.6 vs 49.2	0.44	1.28	Large
TIBC (ug/dL)	386.4 vs 388.2	0.18	0.82	Large
Transferrin sat. (%)	12.4 vs 12.8	0.36	1.14	Large

Table 2 confirms large effect sizes (Cohen's $d > 0.80$) for all haematological parameters at Day 90 in the supplement group versus control. The effect size trajectory from Day 45 (medium effects, $d=0.36-0.52$) to Day 90 (large effects, $d=0.82-1.68$) demonstrates a cumulative, temporally accelerating haematological response to *D. muricata* leaf powder supplementation, consistent with the physiological kinetics of haemoglobin regeneration and ferritin repletion following sustained iron-replete dietary periods. Bonferroni-corrected post-hoc comparisons confirmed statistically significant between-group differences at Day 90 for all parameters (all corrected $p < 0.001$) but only for haemoglobin and serum ferritin at Day 45 (corrected $p < 0.05$), consistent with the observed effect size trajectories.

Discussion

The strong Group x Time interaction effects observed across all haematological parameters (partial eta-squared 0.176-0.246, all $p < 0.001$) provide statistically robust evidence of the efficacy of *D. muricata* leaf powder supplementation at 10 g/day for 90 days in iron-deficient adolescent girls. The comparison with existing literature is informative: a meta-analysis of food-based iron interventions in adolescent girls across 14 randomized trials reported pooled effect sizes for haemoglobin of Cohen's $d=0.64$ (95% CI: 0.42-0.86) [8], compared to the $d=1.42$ observed in the current research at Day 90, suggesting that *D. muricata* leaf powder may deliver superior haematological outcomes relative to other evaluated

food-based iron sources, though direct head-to-head comparisons are required to confirm this inference.

The temporal trajectory of effect sizes from medium at Day 45 ($d=0.44-0.52$) to large at Day 90 ($d=0.82-1.68$) is consistent with the physiological kinetics of haematological recovery. Haemoglobin regeneration requires iron repletion of bone marrow stores before erythropoietic acceleration can be fully expressed, a process that typically requires 6-8 weeks in moderately iron-deficient individuals [9]. The accelerating effect size trajectory observed between Day 45 and Day 90 is therefore mechanistically coherent and suggests that extending the supplementation period beyond 90 days might yield further incremental improvements, particularly for serum ferritin, which serves as the primary indicator of iron store replenishment rather than functional iron status [10].

The partial eta-squared values (0.176-0.246) classify the Group x Time interaction effects as medium-to-large by the conventional thresholds (0.01 small, 0.06 medium, 0.14 large) established for this metric [11]. This contextualizes the statistical significance in terms of practical importance: the observed Group x Time interaction explains between 17.6% and 24.6% of the total variance in haematological trajectories, which represents a substantial treatment-attributable component given the heterogeneity typical of clinical nutritional investigations. Agreement with this interpretation is found in comparably rigorous randomized designs evaluating iron-enriched bread [12] and legume-based iron supplements [13] in adolescent populations, where partial eta-squared values of 0.12-0.22 have been reported for haematological outcomes.

Statistical methodology note

The choice of repeated-measures ANOVA over independent-samples comparisons at each timepoint was deliberate and methodologically appropriate for this research design. Repeated-measures designs exploit within-subject correlations across timepoints, yielding greater statistical power for detecting Group x Time interaction effects than cross-sectional comparisons while controlling for individual baseline differences [14]. The confirmation of sphericity (Mauchly's $W>0.90$ for all parameters) validated the use of uncorrected degrees of freedom; where sphericity is violated, Greenhouse-Geisser or Huynh-Feldt correction should be applied, and future reports of similar designs should explicitly report Mauchly's test results as part of the statistical methods documentation [15].

Conclusion

This research establishes, through rigorous randomized controlled design and full statistical reporting including repeated-measures ANOVA, partial eta-squared, and Cohen's d , that *Digera muricata* leaf powder supplementation at 10 g/day for 90 days produces clinically meaningful and statistically large-effect improvements in haemoglobin ($d=1.42$), serum ferritin ($d=1.68$), serum iron ($d=1.28$), TIBC ($d=0.82$), and transferrin saturation ($d=1.14$) in iron-deficient adolescent girls. These findings strengthen the evidence base for food-based iron interventions using *D. muricata* and provide the statistical parameters effect sizes and partial eta-squared values needed for power calculation in future investigations and meta-analytic synthesis. Future research should investigate minimum effective supplementation periods, optimal dose-response relationships, and comparative efficacy against pharmacological iron-folic acid supplementation in larger, multi-site randomized trials.

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