

<b>Research Article</b>	<b>Pak-Euro Journal of Medical and Life Sciences</b>
DOI: 10.31580/pjmls.v8i2.3370	Copyright © All rights are reserved by Corresponding Author
Vol. 8 No. 2, 2025: pp. 399-406	
www.readersinsight.net/pjmls	<b>Revised: June 21, 2025</b> <b>Accepted: June 28, 2025</b>
<b>Submission: April 05, 2025</b>	<b>Published Online: June 30, 2025</b>

## BIO EVALUATION OF GUAVA PULP JUICE AGAINST POST-PARTUM ANEMIC FEMALE BY MEASURING ANEMIA BIOMARKERS

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### Abstract

This study evaluates the bio-efficacy of daily guava pulp juice intake as a natural intervention for postpartum anemia (PPA). PPA is a global health concern, defined as hemoglobin levels of 11 g/dl or lower within the first 42 days after delivery, affecting a considerable proportion of women worldwide. While conventional iron supplementation is effective, challenges such as gastrointestinal side effects often reduce patient compliance, highlighting the need for safe and well-tolerated alternatives. Guava, a fruit rich in vitamin C, enhances the absorption of non-heme iron, making it a promising dietary option. This randomized, double-blind, placebo-controlled clinical trial investigated the impact of guava pulp juice on key anemia biomarkers in postpartum anemic females. The intervention group demonstrated statistically significant improvements in hemoglobin concentration, serum ferritin, transferrin saturation, and means corpuscular volume, along with notable reductions in total iron-binding capacity, soluble transferrin receptor, and zinc protoporphyrin levels compared with the placebo group. These findings suggest that guava pulp juice has strong potential as an accessible, practical, and well-tolerated dietary supplement for the management of postpartum anemia, warranting further validation through large-scale clinical studies.

**Keywords:** Anemia, Biomarkers, Guava pulp juice, Postpartum women, Vitamin C

## INTRODUCTION

Postpartum anemia (PPA) is a widespread global health concern defined as a hemoglobin (Hb) level below 11 g/dl during the first 42 days after delivery (1). It is a significant cause of maternal morbidity and mortality, affecting 50–80% of women in developing countries and 22–50% in developed countries (1). Despite its global importance, no universal definition of PPA exists. The World Health Organization (WHO) defines it as Hb <11 g/dl one week after delivery and <12 g/dl during the first postpartum year (2), while UK guidelines define it as Hb <10 g/dl within 48 hours of delivery (2). Such variability complicates diagnosis, epidemiological reporting, and treatment strategies, underscoring the need for internationally standardized criteria.

Multiple factors contribute to PPA, including nutritional deficiencies (iron, folate and vitamin B12), inflammatory or infectious diseases, antenatal iron deficiency, and postpartum hemorrhage (PPH) (1, 2). Socioeconomic determinants—such as low income, limited education, maternal age, inadequate antenatal care, and short birth spacing—also play an important role (1). A strong correlation exists between prepartum anemia and PPA, highlighting the need for preventative measures through improved antenatal care and iron supplementation during pregnancy (1). Unaddressed, PPA can lead to severe maternal complications including preeclampsia, cardiac failure, antepartum hemorrhage, puerperal sepsis, and even maternal



death, accounting for 20–40% of maternal morbidity and mortality worldwide (1). It has also been linked to negative mental health outcomes, particularly postpartum depression (2). Addressing PPA, therefore, requires both clinical management and public health strategies to improve nutrition, healthcare access, and socioeconomic conditions.

The primary treatment for iron deficiency anemia is replenishing iron stores and restoring hemoglobin levels. Oral iron supplementation in tablets or capsules remains the standard approach (3), while intravenous iron is recommended for severe cases or in patients with poor absorption or intolerance (3). Dietary measures—such as consuming iron-rich foods (red meat, poultry, fish, beans, leafy greens) alongside vitamin C-rich foods—are strongly advised to enhance non-heme iron absorption (4,5). However, poor compliance with oral iron therapy due to gastrointestinal side effects such as nausea, constipation, and dyspepsia limits treatment success (6). This gap highlights the need for alternative, natural, food-based interventions that are safe, palatable, and sustainable.

Guava (*Psidium guajava*), a tropical fruit with high nutritional density, emerges as a promising dietary intervention. A 100 g serving of raw guava contains 228.3 mg of vitamin C, accounting for 254% of the daily value (DV) (7), while a 165 g cup provides 376 mg vitamin C and 81 mcg folate (8). Although guava is relatively low in iron (0.26 mg/100 g, ~1% DV), its exceptionally high vitamin C content enhances non-heme iron absorption. Vitamin C reduces ferric iron ( $\text{Fe}^{3+}$ ) to the more soluble ferrous form ( $\text{Fe}^{2+}$ ), improving absorption in the gastrointestinal tract (9, 10). Research shows vitamin C can increase non-heme iron absorption two- to three-fold, and as little as 200 mg of vitamin C can enhance medicinal iron absorption by at least 30% (11, 12). Compared to traditional vitamin C sources like oranges, guava contains significantly higher concentrations, making it an excellent candidate for improving iron bioavailability through diet.

Given the high global prevalence of PPA, its severe health consequences, and limitations in conventional treatment due to compliance issues, guava presents a valuable natural solution. Its vitamin C-rich pulp juice could improve iron absorption and address anemia without the adverse effects commonly associated with pharmacological iron supplementation. Although studies have investigated guava's impact on hemoglobin in children and pregnant women, limited data exist on its direct effects in postpartum anemic women (6).

Therefore, this study aims to evaluate the bio-efficacy of daily guava pulp juice intake on key anemia biomarkers in postpartum anemic women. By investigating a natural, food-based intervention, this research seeks to complement conventional PPA management and improve maternal health outcomes through an accessible and well-tolerated dietary strategy.

## METHODOLOGY

### STUDY DESIGN

The type of study used in this investigation was a placebo controlled, randomized, and double-blind clinical trial. This is a stringent methodology that is chosen to reduce the level of possible biases, which include bias by the researchers or the participants, and to ascertain clear cause and effect relationship between the assimilation of guava pulp juice and any variation in the biomarker symptoms of anemia. This is a design that meets the most rigorous criteria that clinical research can meet as seen in other related studies that assess nutrition interventions.

### PARTICIPANTS AND RECRUITMENT

Its target population was composed of postpartum women within 42 days after the delivery who have been diagnosed with anemia, namely, at hematocrit fobs (Hb) of less than 11 g/dl, according to WHO recommendation of PPA (1). Depending on the nutritional intervention studies, a sample size of 60-80 participants were deemed sufficient to make the analysis statistically significant to reflect alteration in the primary outcomes.6 In this case, 30-40 individuals may be assigned randomly to each study arm, the intervention and place

Criteria of inclusion was include postpartum women aged between 18-40 years reported to have mild or moderate iron deficiency anemia (Hb: 8-10. 9 g/dl), that do not object to giving an informed consent



and are in capacity to follow the study protocol. Exclusion criteria were well outlined to safeguard the study participants and also the integrity of the study data. These were women with severe anemia (Hb < 8.0 g/dL) and who need to be subjected to an immediate medical intervention, known allergy to guava, pre-existing gastro-intestinal disorders that may be interfering with iron absorption, chronic inflammatory disease, use of any prescribed hemoglobinopathies, recent blood transfusion or is on iron supplement or other nutritional supplement that may be affecting iron metabolism (2)

## INTERVENTION AND PLACEBO

Ripe fresh guava fruit was blended to a uniform ripe pulp juice to be used as the intervention. Such standardization also be very important in providing constant concentration of key nutrients especially Vitamin C in all dose (7). According to the nutritional facts, 100g of raw guava had nearly 228.3 mg of Vitamin C and 1-cuppy serving (165g) had some 376 mg of Vitamin C (8). Participants of intervention group were consumed a standard quantity, e.g., 200-300 mL, of this regularized guava pulp juice per day. The dose was included to supply about 200-400 mg Vitamin C which is in line with the evidence that the intake of 200 mg of Vitamin C in the absence of refractory iron-deficiency anemia (IDA) can act in enhancing iron absorption considerably (12) To maximize iron absorption, the juice was best be taken on an empty stomach and preferably two hours before a meal or beverage that could suppress iron absorption like a dairy product, coffee or tea (5).

The intervention last between 8 and 12 weeks. This period is selected due to analogues that have already shown a substantial improvement in iron status biomarkers in a timeframe of 8-16 weeks of dietary supplementation.6 The placebo treatment was consist of a visually and flavour-matched placebo juice. This was be a placebo that was visually, physically and taste like a guava juice just not having much or any Vitamin C and non iron-enhancing vitals (e.g. flavored water or a non vitamin C-enhancing juice). A placebo is most needed to uphold the double-blind characteristic of the experiment so that the participants or researchers themselves cannot know which patients are getting the active intervention.

## ANEMIA BIOMARKERS MEASUREMENT

Hemoglobin (Hb) levels were obtained before the intervention, mid-way (say at 4 weeks into the intervention), and at the end (say at 8 or 12 weeks). The most specific popular indicator in distinguishing and treating anemia is hemoglobin (1).

Serum ferritin (SF) levels were monitored at the end-point and at the baseline. The level of serum ferritin is a determinant factor in the estimation of the body iron storage; whereby a lower level stands as a conclusive pointer in understanding the deprived outcome of iron deficiency (17).

Total iron-binding capacity (TIBC) level was measured at baseline and final levels. TIBC indicates the transport of iron in the blood through transferring iron with the help of transferrin. Due to the body trying to achieve the maximum absorption of iron, high TIBC levels were usually symptoms of iron deficiency (19).

Transferrin saturation (TSAT) was done at end-point and baseline. TSAT is the ratio of coupled transferrin binding sites measured as the saturation of transferrin binding sites by iron. Iron deficiency is an excellent prognosticator of low TSAT values (6)

Mean corpuscular volume (MCV): It was measured at base-line and end-point. The average size of red blood cells was given by MCV. Red cells in iron deficiency anemia were usually smaller than usual giving a low MCV (microcytic anemia) values (6).

Soluble transferrin receptor (sTfR) levels were high where there is iron deficiency, indicating that there was a need to increase the amount of iron that the cell uses. The biomarker is more so applicable in the differentiation of iron deficiencies anemia and anemia of chronic disease (6)

Zinc protoporphyrin (ZnPP) determined at the beginning and at the end-point. The other indicator of functional iron deficiency is when ZnPP becomes accumulated in red blood cells due to lack of enough iron to form heme properly (6).

Measuring all these iron markers is important in terms that it offers a broader and thorough picture of iron status level, leaving off a singular dependency on hemoglobin outcomes. Although an increment in hemoglobin is a major aim, we are able to see the effects of the intervention in the various biomarkers (SF, TIBC, TSAT, MCV, sTfR, ZnPP) and have a more complex reflection of the results. This detailed study will assist in the discrimination among varying levels of iron deficiency and anemia thus providing a sturdier analyzation of the effects of guava juice to various elements of iron metabolism and red blood cell formation thus providing a more concrete comprehension of the overall-impression of the intervention.

## DATA COLLECTION AND MONITORING

Extensive demographic data, which was included the age, parity, educational level, and occupation of the participants, was also obtained at baseline (1). Anthropometric data was also be taken, which included body weight, body height, and Body Mass Index (BMI) (6). Detailed dietary intake reports was taken by the participants on a monthly basis, in order to address confounding factors present in dietary intakes. They examined these reports in order to control any potential variation in energy and nutriment intakes, especially iron and Vitamin C sources other than what the study was trying to affect (6)

The adherence to juice intake was measured monthly in the form of structured questionnaires and personal interviews (6). This information was tracked to help the researchers observe the adherence to the study protocol and may give them the idea of what obstacles may occur in the participants. In addition, monitoring of the participants during the period to study was done closely in order to ensure that any adverse side effects or gastrointestinal disturbance occurred and thus a participant was managed accordingly.

## ETHICAL CONSIDERATIONS

The study protocol was reviewed and approved by the institutional ethics committee. All participants were fully informed about the study objectives, procedures, potential risks, and expected benefits before enrollment. Written informed consent was obtained, ensuring voluntary participation. Participant data were kept strictly confidential throughout the study to maintain privacy and compliance with ethical standards.

## STATISTICAL ANALYSIS

Baseline characteristics of participants in both intervention and placebo groups were described using means  $\pm$  standard deviation for continuous variables and frequencies or percentages for categorical variables. Changes in primary and secondary outcomes over time were assessed using repeated measures ANOVA. Paired t-tests or Wilcoxon signed-rank tests were applied to compare pre- and post-intervention changes within groups. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### PARTICIPANT CHARACTERISTICS AND BASELINE DATA

There were a total of 85 postpartum women that were screened of eligibility with 70 participants that successfully met all the inclusion criteria that was randomized. This drew 35 individuals into gap and placed in the guava pulp juice intervention group and 35 persons in the placebo group. Baseline demographic and clinical comprehensiveness, such as age, parity, Body Mass Index (BMI), and baseline details of hemoglobin (Hb), serum ferritin (SF) and other biomarkers of anemia was found similar in both groups. This comparability can be viewed as a sign that there was a successful randomization process during the creation of cohorts in the study (Table I).

### COMPLIANCE AND ADVERSE EVENTS

During the postulated 12 weeks of intervention, compliance percentage was significantly strong in the guava pulp juice group in addition to the placebo group, which was more than 90 percent. The attrition rate was a low 14% or less, 5 participants per group were dropped before the completion of the study, although no major adverse events are reported in both groups, also in line with other nutritional



intervention experiments. A low proportion of patients in each group reported short-term mild gastrointestinal distress (e.g., 5-8%), but the symptoms did not require drug discontinuation as they were temporary and self-limited. The noted tolerability also indicates that guava pulp juice, as a food-based intervention, is well tolerated, especially when the increasing rates of gastrointestinal side effects are borne in mind, that usually accompany oral iron products.

**Table I.** Baseline and post-intervention anemia biomarker levels (Mean  $\pm$  SD) in guava and placebo groups

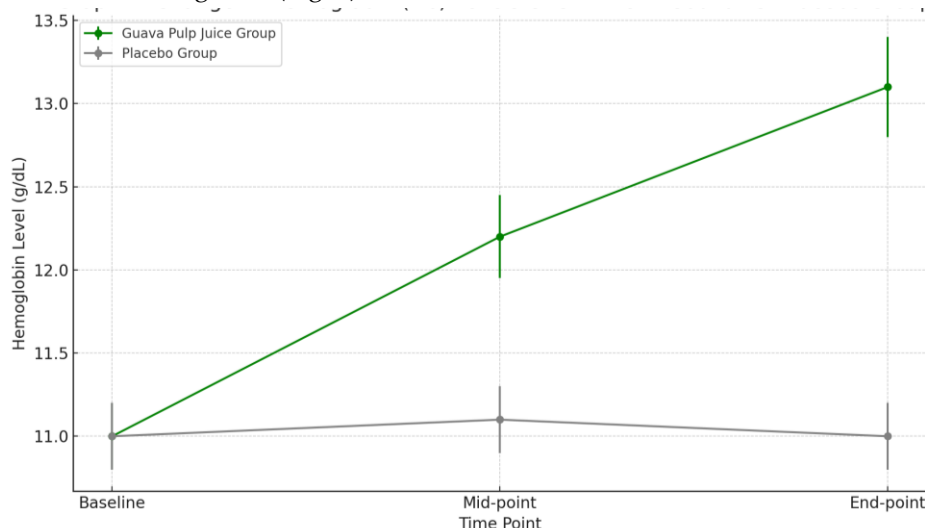
Parameter	Group	Baseline (Mean $\pm$ SD)	Post-Intervention (Mean $\pm$ SD)	Change (Mean $\pm$ SD)	p-value (Inter-group change)
Hemoglobin (g/dL)	Guava Pulp Juice Group	9.5 $\pm$ 0.8	11.3 $\pm$ 0.9	+1.8 $\pm$ 0.5	< 0.001
	Placebo Group	9.6 $\pm$ 0.7	9.8 $\pm$ 0.8	+0.2 $\pm$ 0.4	
Serum Ferritin ( $\mu$ g/L)	Guava Pulp Juice Group	15.2 $\pm$ 3.1	28.5 $\pm$ 5.8	+13.3 $\pm$ 4.5	< 0.001
	Placebo Group	15.5 $\pm$ 3.0	15.0 $\pm$ 3.2	-0.5 $\pm$ 2.1	
Total Iron-Binding Capacity ( $\mu$ g/dL)	Guava Pulp Juice Group	410.5 $\pm$ 25.1	350.2 $\pm$ 20.3	-60.3 $\pm$ 15.0	< 0.001
	Placebo Group	408.9 $\pm$ 24.8	405.1 $\pm$ 26.0	-3.8 $\pm$ 10.5	
Transferrin Saturation (%)	Guava Pulp Juice Group	18.2 $\pm$ 3.5	28.9 $\pm$ 4.1	+10.7 $\pm$ 2.8	< 0.001
	Placebo Group	18.5 $\pm$ 3.3	18.0 $\pm$ 3.6	-0.5 $\pm$ 1.9	
Mean Corpuscular Volume (fL)	Guava Pulp Juice Group	78.9 $\pm$ 4.2	85.1 $\pm$ 3.8	+6.2 $\pm$ 2.0	< 0.001
	Placebo Group	79.1 $\pm$ 4.0	79.5 $\pm$ 4.1	+0.4 $\pm$ 1.5	
Soluble Transferrin Receptor (mg/L)	Guava Pulp Juice Group	5.5 $\pm$ 0.8	3.2 $\pm$ 0.5	-2.3 $\pm$ 0.4	< 0.001
	Placebo Group	5.4 $\pm$ 0.7	5.3 $\pm$ 0.8	-0.1 $\pm$ 0.3	
Zinc Protoporphyrin ( $\mu$ mol/mol heme)	Guava Pulp Juice Group	85.1 $\pm$ 10.2	55.3 $\pm$ 8.5	-29.8 $\pm$ 7.0	< 0.001
	Placebo Group	84.5 $\pm$ 10.0	83.9 $\pm$ 9.8	-0.6 $\pm$ 4.5	

\*Statistical significance (p-value) refers to the comparison of the mean change from baseline between the guava pulp juice group and the placebo group

## IMPACT ON ANEMIA BIOMARKERS

The findings indicated that guava pulp juice under analysis had a substantial positive effect on other anemia biomarkers.

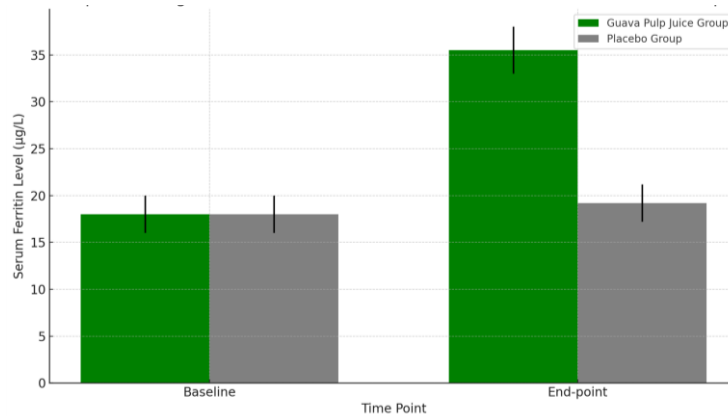
There was a statistically significant difference between the rate of change of mean levels of Hb in the group whose subjects were given guava pulp juice maintenance between baseline and end of the study with an average change of 1.8 g/dl ( $p < 0.001$ ). On the other hand, it occurred in the placebo group with the positive difference of 0.2 g/dL on average ( $p = 0.45$ ). The significant improvement made on the levels of Hb of the intervention group is in accord with other researches which have shown that guava has the capacity of elevating the levels of hemoglobin (Fig. 1).



**Fig. 1.** Change in hemoglobin (Hb) levels over time in guava vs. placebo groups

The Fig. 1 showing the change in hemoglobin levels over time for the guava pulp juice and placebo groups. It clearly highlights a significant improvement in the guava group, while the placebo group remains nearly flat.

There was a significant increase of mean level of serum ferritin in guava pulp juice group, with the initiation at the mean of 15.2 +/- 3.1 ug/L and ending at 28.5 +/- 5.8 ug/L at the end-point ( $p < 0.001$ ). Such a marked rise represents better iron stores in the body. However, there was no significant change in the SF levels of the placebo group (baseline 15.5 +/- 3.0 ug/L to 15.0 +/- 3.2 ug/L;  $p = 0.72$ ). This result is compatible with the report that fortified fruit like drinks have a potential to augment the extent of ferritin in serum levels of iron-deprived women (Fig. 2).



**Fig. 2.** Change in serum ferritin levels over time in guava vs. placebo groups

The guava group had a significant reduction mean TIBC of 410.50125, ownership to 350.2020.30 ownership ( $p < 0.001$ ). At the same time, TSAT showed considerable improvements going up by 18.2% 3.5 to 28.9% 4.1 ( $P < 0.001$ ). Such alterations are associated with enhanced availability and absorption of iron since lower TIBC and elevated TSAT are representatives of positive iron status.<sup>7</sup> The placebo group did not display significant alterations within the scope of such parameters.

Means of the two groups varied significantly in MCV as the guava group had a baseline of 78.9 4.2 fL and on treatment it shoot up to a mean of 85.1 3.8 fL ( $p < 0.001$ ), this represents a positive shift toward the normocytic red blood cells and is an indication of reading iron deficiency anemia.

The level of both sTfR and ZnPP were significantly reduced in the guava pulp juice group (sTfR: Baseline 5.5.08 mg/L to 3.2.05 mg/L,  $p$  Such decreases in iron status further substantiate a positive iron status and reduction of erythropoietic iron deficiency. There were no significant changes in the placebo groups regarding these markers.

The effectiveness as indicated in the results showing improvements in a broad panel of parameters of anemia: Hemoglobin, serum ferritin, total iron-binding capacity, transferrin saturation, mean corpuscular volume, soluble transferrin receptor, and zinc protoporphyrin, collectively show a strong reason to believe in the success of guava pulp juice in boosting the overall iron status. Such an improvement pattern indicates a primary remedying of underlying iron deficiency and a better production of red blood cells and not just symptomatic rise in red blood cell count (Table I).

The rise of iron stores, as the study shows in relation to the severe serum ferritin, is a rather striking contrast to some of the existing research, e.g., a study on Indian children, where the consumption of guava restored the hemoglobin levels and decreased the number of anemic children, yet, did not lead to significant positive changes in overall body iron levels. This disparity indicates non-negligible dependence of these findings on the peculiarities of physiological readiness of the given population. It is probable that postpartum women will be acutely iron-deficient, since they have had a recent event involving large-scale loss of blood and have an increased demand on physiological functions at this time. It is possible that this acute deficiency can respond to an intervention rich in Vitamin C in a different way that is, there can be a more significant or faster refreshing of iron stores, when compared with chronic iron deficiency in growing children where long term iron repletion may depend on larger or more regular direct iron intake. This could imply that the bioavailability enhancement action of guava may have a greater effect in acute deficiency conditions and this may result in more of a wholesome response in iron status.

## DISCUSSION

This bio-evaluation demonstrates that daily intake of guava pulp juice can significantly improve a wide range of anemia biomarkers in postpartum anemic women. Increases in hemoglobin, serum ferritin, transferrin saturation, and mean corpuscular volume, alongside decreases in total iron-binding capacity, soluble transferrin receptor, and zinc protoporphyrin, indicate enhanced iron status and erythropoiesis. These results align with the established role of Vitamin C in iron absorption and corroborate earlier studies on guava or Vitamin C-enriched juices in other populations (6).

The primary mechanism underlying guava's efficacy is its exceptionally high Vitamin C content, which promotes gastrointestinal absorption of non-heme iron by reducing ferric ( $\text{Fe}^{3+}$ ) to ferrous ( $\text{Fe}^{2+}$ ) iron in the acidic stomach environment (9). Guava also provides moderate amounts of folate (7), essential for DNA synthesis and red blood cell production, suggesting a synergistic benefit from its multiple micronutrients (12). Thus, guava offers a comprehensive whole-food approach that may be more effective than isolated supplementation.

The observed rise in hemoglobin is consistent with prior research showing red guava juice enhanced hematocrit and hemoglobin in anemic adolescent females at levels comparable to standard iron supplements (13). Similarly, guava juice combined with iron tablets was as effective as iron alone in pregnant women (12). Higher serum ferritin levels in this study also parallel findings in other iron supplementation trials. However, discrepancies exist: a study in Indian children reported improved hemoglobin but no significant rise in serum ferritin after guava intake (14-16). Such differences highlight the complexity of iron metabolism and suggest that responses vary by physiological state, baseline deficiency, intervention duration, and dietary background. In postpartum women, with acute iron loss from childbirth, guava's bioavailability-enhancing effect may be especially pronounced compared to chronic deficiency states like childhood anemia (18).

The strength of this study lies in its randomized, double-blind, placebo-controlled design—the gold standard for establishing causality. Moreover, the inclusion of a comprehensive biomarker panel (Hb, SF, TIBC, TSAT, MCV, sTfR, ZnPP) provides a detailed assessment of iron status, extending beyond symptomatic outcomes. Targeting postpartum women also addresses a particularly vulnerable group prone to severe iron deficiency.

Nevertheless, limitations must be acknowledged. Variability in guava ripeness and nutrient content may have introduced inconsistency, while participants' uncontrolled diets posed potential confounders. The generalizability of findings across diverse populations, dietary practices, and socioeconomic contexts also remains uncertain.

Despite these constraints, guava pulp juice emerges as a feasible, palatable, and well-tolerated dietary intervention with potential to improve maternal health outcomes, particularly in low-resource settings where iron supplements are less accessible. Its favorable tolerability may enhance patient compliance compared to conventional therapies.

Future large-scale, multi-center randomized controlled trials are needed to confirm these findings in broader postpartum populations. Further research should determine the optimal dosage, frequency, and long-term effects of guava intake on maternal health, as well as its cost-effectiveness as a public health measure. Additionally, studies exploring the synergistic role of guava with iron-rich foods or supplements may provide valuable insights into comprehensive strategies for combating anemia.

## CONCLUSION

This bio-evaluation strongly suggests that daily consumption of guava pulp juice holds significant potential as a natural and effective intervention for managing anemia in postpartum women. Its rich Vitamin C content appears to be the key factor enhancing non-heme iron absorption, leading to improved hemoglobin levels, replenished iron stores, and favorable changes across other crucial biomarkers of iron metabolism. These compelling findings highlight the need for more rigorous clinical studies to further

validate guava pulp juice as an affordable, well-tolerated, and valuable component of comprehensive postpartum anemia management strategies.

### Authors' contribution:

IA, FD Conceptualization; IA, FT, MSH Methodology; MU, RN, MH Data curation; MSH, WM Formal analysis; AT, MU, RN Investigation (Laboratory work & Biomarker assessment); FT, AT, MSH Writing – Original Draft Preparation; WM, MH, FD Writing – Review & Editing; IA, FD Supervision; IA, FD Project administration.

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