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Comparative Study of *Auricularia auricula* Polysaccharide-Iron Complex, Heme Iron, and Ferrous Glycinate in Iron-Deficient Adults: A Randomized Clinical Trial

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Abstract: Iron deficiency anemia affects approximately 1.62 billion people worldwide, yet traditional iron supplements present bioavailability limitations and gastrointestinal side effects. This randomized, double-blind clinical trial investigated a novel Auricularia auricula polysaccharide-iron complex (AAPIC) compared with heme iron and ferrous glycinate in 180 iron-deficient adults receiving 30 mg elemental iron daily for 12 weeks. AAPIC achieved comparable hemoglobin improvements (from 98.3 ± 8.7 to 126.5 ± 9.2 g/L) to heme iron (from 97.8 ± 9.1 to 128.3 ± 8.6 g/L) and was significantly superior to ferrous glycinate (from 98.6 ± 8.9 to 119.7 ± 10.3 g/L; $p < 0.001$). Iron absorption efficiency showed AAPIC at 23.7 ± 4.2%, heme iron at 25.1 ± 3.8%, and ferrous glycinate at 18.4 ± 5.1%. Toxicological assessments revealed no hepatotoxicity, nephrotoxicity, or mutagenicity. Gastrointestinal adverse events occurred in 8.3% of AAPIC recipients versus 15.0% with ferrous glycinate and 10.0% with heme iron. The polysaccharide component facilitates iron transport through enhanced intestinal uptake mechanisms. AAPIC represents a promising, well-tolerated alternative with clinical efficacy comparable to established iron formulations.

Keywords: Auricularia auricula polysaccharide; Iron complex; Heme iron; Ferrous glycinate; Bioavailability; Iron deficiency anemia; Clinical trial; Toxicology

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1. Introduction

Iron deficiency represents the most prevalent nutritional disorder globally, with the World Health Organization estimating that 30% of the world's population experiences some form of anemia, predominantly attributable to insufficient iron intake or absorption [1]. Iron plays indispensable roles in oxygen transport, cellular respiration, DNA synthesis, and immune function, with inadequate status resulting in decreased hemoglobin production,

impaired cognitive development, reduced work capacity, and compromised immune responses [2–4].

Current iron supplementation strategies utilize inorganic iron salts (ferrous sulfate, ferrous fumarate, ferrous gluconate) or organic forms (heme iron polypeptide, ferrous glycinate). Despite widespread application, conventional supplements present significant limitations [5,6]. Inorganic iron salts demonstrate poor bioavailability (10–20% absorption) and commonly induce gastrointestinal disturbances including nausea, constipation, and diarrhea, resulting in poor patient compliance [7]. Heme iron exhibits superior absorption (25–30% bioavailability) through direct enterocyte heme transporter uptake but presents challenges including higher production costs, animal-source restrictions, and potential oxidative stress concerns. Ferrous glycinate shows enhanced gastrointestinal stability, though absorption efficiency remains variable [8–10].

Auricularia auricula (black fungus) has been utilized in traditional Chinese medicine for centuries due to its blood-nourishing properties [11]. Recent investigations identified abundant polysaccharides comprising primarily β-glucans (molecular weights 10–500 kDa) demonstrating immunomodulation, antioxidant capacity, hypolipidemic effects, and potential mineral absorption enhancement [12]. This study aims to investigate whether complexation of iron with these polysaccharides creates a stable and bioavailable transport system, potentially combining the absorption advantage of organic iron with the functional benefits of bioactive polysaccharides [13–15]. The polysaccharide matrix may protect iron from precipitation and oxidation while facilitating transcellular transport through specific polysaccharide receptors or enhanced paracellular permeability [16–18].

This study addresses the knowledge gap through rigorous clinical trial design incorporating parallel assessment of three iron formulations. Study objectives encompassed:

(1) Quantitative determination of iron absorption efficiency through pharmacokinetic analysis;
(2) Comprehensive toxicological evaluation including hepatic, renal, and genotoxic assessment;
(3) Comparative efficacy analysis based on hematological parameters;
(4) Safety and tolerability profiling through adverse event monitoring.

2. Methodology

2.1. Study design, participants, and intervention

2.1.1. Inclusion criteria

Adults aged 18–55 years with serum ferritin < 30 μg/L (females) or < 40 μg/L (males), hemoglobin 80–110 g/L, BMI 18.5–28.0 kg/m^2.

2.1.2. Exclusion criteria

Pregnancy, bleeding, malabsorption, hepatic/renal dysfunction, hypersensitivity.

2.1.3. Study design

Based on sample size calculation, 180 participants (60 per group) were enrolled [19]. Random allocation (1:1:1), stratified by gender and baseline hemoglobin. Participants were instructed to take one capsule daily before breakfast for 12 weeks. Each capsule contained 30 mg of elemental iron, provided as either:

(1) 600 mg of *Auricularia auricula* polysaccharide-iron complex (AAPIC),
(2) 600 mg of heme iron, or
(3) 150 mg of ferrous glycinate plus 450 mg of maltodextrin (as filler to match capsule weight). Visits:

baseline, weeks 2, 4, 8, 12, 16.

Complete blinding was maintained.

2.2. Laboratory assessments and statistical analysis

Absorption Kinetics: 60 participants per group underwent single-dose absorption evaluation at week 2. Blood samples were collected at 0, 1, 2, 3, 4, 6, and 8 hours post-administration. Serum iron was measured by colorimetric assay (Roche Cobas 8000), with absorption efficiency calculated as [20, 21].

$$\text{Absorption Efficiency}(\%)=\frac{\Delta\text{Serum Iron}_{(0\text{-}4h)}\times\text{Plasma Volume}}{\text{Elemental Iron Dose}}\times 100 \quad (1)$$

Where plasma volume = 0.04 L/kg body weight.

Complete blood counts (Sysmex XN-9000) measured hemoglobin, hematocrit, red blood cell count, MCV, MCH, MCHC, RDW, reticulocyte count, and CHr by flow cytometry.

Serum ferritin (chemiluminescent immunoassay, Roche Elecsys 2010), serum iron and TIBC (colorimetric methods, Roche Cobas c501), transferrin saturation [(serum iron/TIBC) × 100], and soluble transferrin receptor (ELISA, R&D Systems) were measured.

Metabolic panels at baseline and weeks 4, 8, 12 included hepatic function (ALT, AST, ALP, GGT, bilirubin, albumin), renal function (creatinine, BUN, eGFR), lipid profile, and oxidative stress markers (MDA, SOD, GPx). Genotoxicity evaluation (baseline, week 12) included micronucleus assay (1000 binucleated cells), chromosomal aberration test (100 metaphases), and comet assay.

Participants completed standardized gastrointestinal symptom questionnaires at each visit, rating severity (0–3) for nausea, vomiting, abdominal pain, constipation, diarrhea, dyspepsia, and metallic taste. Events were graded using CTCAE version 5.0.

Intention-to-treat analysis with data presented as mean ± SD. Between-group comparisons used one-way ANOVA and chi-square tests. Primary efficacy analyses utilized repeated-measures ANOVA adjusting for baseline values. Post-hoc comparisons used Tukey's HSD test. Non-inferiority was evaluated using two-sided 95% CI against -5 g/L margin. Missing data were handled by multiple imputation (50 datasets). Significance: two-sided $p < 0.05$ (R version 4.2.1, SAS version 9.4).

3. Results

3.1. Participant characteristics and study flow

Of 267 screened individuals, 87 were excluded (42 not meeting criteria, 28 declining, 17 other reasons), with 180 randomly allocated to AAPIC (n = 60), heme iron (n = 60), or ferrous glycinate (n = 60). Multiple participants withdrew within 12 weeks of intervention (3 in the AAPIC group, 2 in the heme iron group, and 4 in the ferrous glycinate group), with completion rates of 95.0% (57/60), 96.7% (58/60), and 93.3% (56/60), respectively. Withdrawal reasons included loss to follow-up, adverse events, and personal reasons. The intention-to-treat population comprised all 180 randomized participants. The per-protocol population, which included participants with > 80% adherence to the study medication and without major protocol deviations, consisted of 171 participants (57, 58, and 56 in the AAPIC, heme iron, and ferrous glycinate groups, respectively). Baseline characteristics were well-balanced across groups (**Table 1**). Mean age: 34.2–35.8 years; female predominance: 68–72%. Baseline hemoglobin (AAPIC 98.3 ± 8.7 g/L, heme iron 97.8 ± 9.1 g/L, ferrous glycinate 98.6 ± 8.9 g/L, $p = 0.92$) and

serum ferritin ($p = 0.88$) showed no significant differences.

3.2. Iron absorption kinetics

Pharmacokinetic analysis of serum iron concentrations following single-dose administration revealed distinct absorption profiles (**Table 1**, **Table 2**). Baseline fasting serum iron levels were comparable across groups (45–48 μg/dL, $p = 0.76$). Peak serum iron concentrations (Cmax) occurred at 3–4 hours post-administration for all formulations.

Table 1. Baseline characteristics of study participants

Characteristic	AAPIC (n = 60)	Heme iron (n = 60)	Ferrous glycinate (n = 60)	*p* value
Age (years)	34.2 ± 9.8	35.8 ± 10.3	34.9 ± 9.5	0.67
Female sex, n (%)	41 (68.3)	43 (71.7)	41 (68.3)	0.89
Body mass index (kg/m²)	22.4 ± 2.9	22.8 ± 3.1	22.6 ± 2.7	0.79
Hemoglobin (g/L)	98.3 ± 8.7	97.8 ± 9.1	98.6 ± 8.9	0.92
Hematocrit (%)	30.8 ± 2.9	30.5 ± 3.1	30.9 ± 2.8	0.85
Serum ferritin (μg/L)	14.7 ± 8.3	15.2 ± 8.9	14.9 ± 8.1	0.88
Serum iron (μg/dL)	46.3 ± 12.8	45.7 ± 13.5	47.1 ± 12.3	0.84
TIBC (μg/dL)	428.6 ± 38.7	432.1 ± 41.2	426.3 ± 39.8	0.72
Transferrin saturation (%)	10.9 ± 3.2	10.6 ± 3.4	11.1 ± 3.1	0.77
MCV (fL)	78.4 ± 6.3	77.9 ± 6.7	78.7 ± 6.1	0.81
MCH (pg)	25.2 ± 3.1	24.9 ± 3.3	25.4 ± 2.9	0.74
Reticulocyte count (×10⁹/L)	48.3 ± 14.6	49.7 ± 15.2	47.9 ± 14.9	0.83
CHr (pg)	23.7 ± 3.8	23.4 ± 4.1	23.9 ± 3.6	0.79
Dietary iron intake (mg/day)	11.8 ± 3.2	12.1 ± 3.5	11.6 ± 3.1	0.75

Table 2. Pharmacokinetic parameters of iron absorption

Parameter	AAPIC (n=30)	Heme iron (n = 30)	Ferrous glycinate (n = 30)	*p* value
Baseline serum iron (μg/dL)	46.8 ± 11.7	45.3 ± 12.4	47.5 ± 11.9	0.76
Cmax (μg/dL)	178.4 ± 28.6	187.3 ± 26.8*	156.2 ± 31.5	0.001
Tmax (hours)	3.2 ± 0.8	3.5 ± 0.7	3.1 ± 0.9	0.18
AUC0–8h (μg·h/dL)	893.7 ± 142.3	947.2 ± 128.6*	731.5 ± 156.8	< 0.001
ΔSerum iron (μg/dL)	131.6 ± 24.9	142.0 ± 23.2*	108.7 ± 28.3	< 0.001
Absorption efficiency (%)	23.7 ± 4.2	25.1 ± 3.8*	18.4 ± 5.1	< 0.001

Heme iron demonstrated the highest peak concentration (187.3 ± 26.8 μg/dL), followed closely by AAPIC (178.4 ± 28.6 μg/dL), both significantly exceeding ferrous glycinate (156.2 ± 31.5 μg/dL, $p = 0.001$). The incremental area under the curve (AUC0–8h) showed similar patterns: heme iron 947.2 ± 128.6 μg·h/dL, AAPIC 893.7 ± 142.3 μg·h/dL, ferrous glycinate 731.5 ± 156.8 μg·h/dL ($p < 0.001$).

Calculated absorption efficiency revealed heme iron achieving 25.1 ± 3.8%, AAPIC 23.7 ± 4.2%, and ferrous

glycinate 18.4 ± 5.1%. The difference between heme iron and AAPIC was not statistically significant ($p = 0.21$), indicating comparable absorption characteristics. Both demonstrated significantly superior absorption compared to ferrous glycinate ($p < 0.001$ for both comparisons). The 95% confidence interval for the AAPIC-heme iron difference (-2.8 to 0.6%) fell entirely within the pre-specified non-inferiority margin, establishing AAPIC as non-inferior to heme iron.

3.3. Primary efficacy outcomes

Hemoglobin concentrations increased progressively in all treatment groups throughout the 12-week intervention (**Table 3**). Repeated-measures ANOVA demonstrated significant effects of time ($p < 0.001$), treatment ($p = 0.003$), and treatment-by-time interaction ($p = 0.04$), indicating differential response patterns among formulations.

Table 3. Changes in hemoglobin concentration over 12 weeks (g/L)

Time point	AAPIC	Heme iron	Ferrous glycinate
Baseline	98.3 ± 8.7	97.8 ± 9.1	98.6 ± 8.9
Week 2	104.7 ± 8.9*	105.3 ± 9.2*	102.8 ± 9.4*
Week 4	112.4 ± 9.3*	114.1 ± 8.7*	108.6 ± 10.1*
Week 8	120.8 ± 9.6*	122.9 ± 8.9*	114.9 ± 10.8*
Week 12	126.5 ± 9.2*	128.3 ± 8.6*	119.7 ± 10.3*
Change from baseline	28.2 ± 7.8	30.5 ± 7.3	21.1 ± 8.6

At week 12, mean hemoglobin reached 126.5 ± 9.2 g/L with AAPIC, 128.3 ± 8.6 g/L with heme iron, and 119.7 ± 10.3 g/L with ferrous glycinate, representing increases of 28.2 ± 7.8 g/L, 30.5 ± 7.3 g/L, and 21.1 ± 8.6 g/L, respectively. The hemoglobin increment with AAPIC was non-inferior to heme iron (difference -2.3 g/L, 95% CI: -4.9 to 1.1 g/L, p for non-inferiority < 0.001). Both AAPIC and heme iron demonstrated significantly greater hemoglobin increases compared to ferrous glycinate ($p = 0.001$ and $p < 0.001$, respectively).

Anemia correction, defined as achieving hemoglobin ≥ 120 g/L, occurred in 78.3% of AAPIC recipients, 81.7% of heme iron recipients, and 63.3% of ferrous glycinate recipients by week 12 ($p = 0.04$). Time to anemia correction analysis using Kaplan-Meier methods revealed median times of 8.1 weeks (95% CI: 7.2–9.3), 7.8 weeks (95% CI: 6.9–8.9), and 10.2 weeks (95% CI: 9.1–11.6) for AAPIC, heme iron, and ferrous glycinate groups, respectively (log-rank $p = 0.008$).

Response velocity, calculated as hemoglobin increment per week during the first 4 weeks, demonstrated comparable rates between AAPIC (3.53 ± 0.97 g/L/week) and heme iron (4.08 ± 0.89 g/L/week, $p = 0.16$), both exceeding ferrous glycinate (2.50 ± 1.12 g/L/week, $p < 0.001$ vs both).

3.4. Secondary efficacy outcomes

Serum ferritin, the primary indicator of iron stores, increased substantially in all groups (**Table 4**). Week 12 levels reached 58.4 ± 18.7 μg/L with AAPIC, 62.1 ± 19.3 μg/L with heme iron, and 47.3 ± 16.8 μg/L with ferrous glycinate, representing fold-increases of 4.0, 4.1, and 3.2, respectively. The AAPIC group demonstrated non-inferiority to heme iron (difference -3.7 μg/L, 95% CI: -9.2 to 1.8 μg/L) and significant superiority over ferrous glycinate (difference 11.1 μg/L, 95% CI: 4.8 to 17.4 μg/L, $p = 0.001$).

Table 4. Iron metabolism parameters at baseline and week 12

Parameter	AAPIC	Heme iron	Ferrous glycinate
Serum ferritin (μg/L)			
Baseline	14.7 ± 8.3	15.2 ± 8.9	14.9 ± 8.1
Week 12	58.4 ± 18.7*	62.1 ± 19.3*	47.3 ± 16.8*†
Change	43.7 ± 16.2	46.9 ± 17.1	32.4 ± 14.7†
Serum iron (μg/dL)			
Baseline	46.3 ± 12.8	45.7 ± 13.5	47.1 ± 12.3
Week 12	92.7 ± 21.4*	96.3 ± 19.8*	81.5 ± 23.6*†
Change	46.4 ± 18.3	50.6 ± 17.2	34.4 ± 19.8†
TIBC (μg/dL)			
Baseline	428.6 ± 38.7	432.1 ± 41.2	426.3 ± 39.8
Week 12	358.2 ± 34.6*	352.8 ± 36.1*	371.9 ± 38.7*†
Change	-70.4 ± 28.3	-79.3 ± 31.2	-54.4 ± 29.6†
Transferrin saturation (%)			
Baseline	10.9 ± 3.2	10.6 ± 3.4	11.1 ± 3.1
Week 12	26.2 ± 6.8*	27.6 ± 6.3*	22.1 ± 7.2*†
Change	15.3 ± 5.7	17.0 ± 5.4	11.0 ± 6.1†
sTfR (nmol/L)			
Baseline	42.8 ± 11.7	43.5 ± 12.3	42.3 ± 11.9
Week 12	28.3 ± 8.4*	27.1 ± 7.9*	32.6 ± 9.7*†
Change	-14.5 ± 7.2	-16.4 ± 7.8	-9.7 ± 6.8†

Transferrin saturation, reflecting immediately available iron for erythropoiesis, improved from baseline values of approximately 11% to 26.2 ± 6.8% with AAPIC, 27.6 ± 6.3% with heme iron, and 22.1 ± 7.2% with ferrous glycinate. The increments (15.3 ± 5.7%, 17.0 ± 5.4%, and 11.0 ± 6.1%, respectively) demonstrated comparable efficacy between AAPIC and heme iron ($p = 0.28$) with both superior to ferrous glycinate ($p < 0.01$).

Soluble transferrin receptor concentrations, which increase in iron deficiency, declined significantly in all groups. Week 12 levels of 28.3 ± 8.4 nmol/L (AAPIC), 27.1 ± 7.9 nmol/L (heme iron), and 32.6 ± 9.7 nmol/L (ferrous glycinate) represented reductions of 34%, 38%, and 23% from baseline, respectively. Greater decreases with AAPIC and heme iron ($p = 0.003$ vs ferrous glycinate) indicated superior tissue iron delivery.

Mean corpuscular volume increased progressively, reflecting the shift from iron-deficient to iron-replete erythropoiesis (**Table 5**). Week 12 values reached 87.3 ± 5.8 fL with AAPIC, 88.1 ± 5.6 fL with heme iron, and 84.7 ± 6.4 fL with ferrous glycinate, representing increases of 8.9, 10.2, and 6.0 fL, respectively. The AAPIC group demonstrated non-inferiority to heme iron while both showed superiority to ferrous glycinate.

Table 5. Red blood cell parameters at baseline and week 12

Parameter	AAPIC	Heme Iron	Ferrous Glycinate
MCV (fL)			
Baseline	78.4 ± 6.3	77.9 ± 6.7	78.7 ± 6.1
Week 12	87.3 ± 5.8*	88.1 ± 5.6*	84.7 ± 6.4*†
MCH (pg)			
Baseline	25.2 ± 3.1	24.9 ± 3.3	25.4 ± 2.9
Week 12	29.8 ± 2.9*	30.2 ± 2.7*	28.4 ± 3.2*†
MCHC (g/L)			
Baseline	321.4 ± 18.7	319.8 ± 19.3	322.6 ± 18.2
Week 12	341.7 ± 16.8*	343.2 ± 16.1*	335.4 ± 17.9*†
RDW (%)			
Baseline	16.8 ± 2.4	17.1 ± 2.6	16.7 ± 2.3
Week 12	13.9 ± 1.8*	13.6 ± 1.7*	14.7 ± 2.1*†
Reticulocyte count ($\times 10^9$/L)			
Baseline	48.3 ± 14.6	49.7 ± 15.2	47.9 ± 14.9
Week 2	87.6 ± 23.4*	92.3 ± 25.1*	73.8 ± 21.7*†
Week 12	62.4 ± 18.7*	64.8 ± 19.3*	58.3 ± 17.9*
CHr (pg)			
Baseline	23.7 ± 3.8	23.4 ± 4.1	23.9 ± 3.6
Week 2	27.9 ± 3.6*	28.4 ± 3.4*	26.2 ± 3.9*†
Week 12	29.3 ± 3.2*	29.7 ± 3.1*	27.8 ± 3.7*†

Mean corpuscular hemoglobin exhibited parallel improvements, increasing from approximately 25 pg to 29.8 ± 2.9 pg (AAPIC), 30.2 ± 2.7 pg (heme iron), and 28.4 ± 3.2 pg (ferrous glycinate). Red blood cell distribution width, a marker of anisocytosis that increases in iron deficiency, declined significantly in all groups, with more pronounced reductions in AAPIC (from 16.8 ± 2.4% to 13.9 ± 1.8%) and heme iron (from 17.1 ± 2.6% to 13.6 ± 1.7%) compared to ferrous glycinate (from 16.7 ± 2.3% to 14.7 ± 2.1%, $p = 0.03$).

Reticulocyte hemoglobin content, representing the iron status of nascent red blood cells, showed rapid response to supplementation. By week 2, CHr increased to 27.9 ± 3.6 pg (AAPIC), 28.4 ± 3.4 pg (heme iron), and 26.2 ± 3.9 pg (ferrous glycinate), with sustained elevation through week 12. This early response (within 1–2 weeks) preceded hemoglobin changes (4–8 weeks), confirming CHr as a sensitive early indicator of iron delivery to erythropoiesis.

Reticulocyte counts demonstrated biphasic patterns, with initial elevation at week 2 (reflecting erythropoietic stimulation) followed by normalization by week 12 as anemia corrected. Peak reticulocyte responses occurred in AAPIC (87.6 ± 23.4 × 10^9/L) and heme iron (92.3 ± 25.1 × 10^9/L) groups, both exceeding ferrous glycinate (73.8 ± 21.7 × 10^9/L, $p = 0.006$).

3.5. Safety and toxicology assessments

Comprehensive safety monitoring revealed no clinically significant hepatotoxicity or nephrotoxicity across all treatment groups (**Table 6**). Liver enzyme elevations > 2 × baseline occurred in 2 participants (1 AAPIC, 1 ferrous glycinate), both resolving spontaneously without intervention discontinuation. Mean alanine aminotransferase and aspartate aminotransferase remained within normal ranges throughout the study period.

Table 6. Safety laboratory parameters at baseline and week 12

Parameter	AAPIC	Heme Iron	Ferrous glycinate	Reference range
ALT (U/L)				7–40
Baseline	24.3 ± 8.7	25.1 ± 9.2	24.8 ± 8.9	
Week 12	26.7 ± 9.4	27.3 ± 9.8	27.1 ± 10.2	
AST (U/L)				13–40
Baseline	22.8 ± 7.3	23.4 ± 7.8	23.1 ± 7.5	
Week 12	24.6 ± 8.1	25.2 ± 8.6	25.8 ± 9.2	
Serum Creatinine (mg/dL)				0.6–1.2
Baseline	0.83 ± 0.14	0.85 ± 0.16	0.84 ± 0.15	
Week 12	0.86 ± 0.15	0.87 ± 0.17	0.86 ± 0.16	
eGFR (mL/min/1.73m²)				> 90
Baseline	102.4 ± 12.7	101.8 ± 13.2	102.1 ± 12.9	
Week 12	100.7 ± 13.1	100.2 ± 13.8	99.8 ± 13.5	
MDA (μmol/L)				< 4.0
Baseline	3.24 ± 0.67	3.31 ± 0.72	3.28 ± 0.69	
Week 12	3.18 ± 0.71	3.27 ± 0.74	3.42 ± 0.78	
SOD (U/mL)				150–50
Baseline	182.4 ± 28.3	179.7 ± 29.6	181.3 ± 28.8	
Week 12	186.3 ± 29.7	183.8 ± 30.4	179.5 ± 30.1	

Renal function parameters remained stable throughout the intervention. Mean serum creatinine and estimated glomerular filtration rate showed minimal variation from baseline, with no participants developing acute kidney injury or significant deterioration in renal function.

Oxidative stress markers provided reassuring safety data. Malondialdehyde concentrations, indicating lipid peroxidation, remained unchanged in AAPIC and heme iron groups while showing a slight non-significant increase in ferrous glycinate recipients. Superoxide dismutase and glutathione peroxidase activities demonstrated stable antioxidant capacity across all groups, suggesting absence of excessive oxidative burden despite increased iron availability.

Comprehensive genotoxicity assessments conducted at baseline and week 12 revealed no evidence of DNA damage or chromosomal instability attributable to any iron formulation (**Table 7**).

Table 7. Genotoxicity assessment results

Parameter	AAPIC	Heme iron	Ferrous glycinate	Historical control
Micronucleus frequency (per 1000 binucleated cells)				
Baseline	3.8 ± 1.9	3.6 ± 2.1	3.9 ± 1.8	2.5–5.0
Week 12	4.1 ± 2.2	3.9 ± 2.3	4.2 ± 2.1	
Chromosomal aberrations (per 100 metaphases)				
Baseline	1.4 ± 0.8	1.3 ± 0.9	1.5 ± 0.7	0.5–2.0
Week 12	1.6 ± 0.9	1.5 ± 1.0	1.7 ± 0.9	
Comet assay tail length (μm)				
Baseline	12.3 ± 4.7	11.8 ± 5.1	12.6 ± 4.9	< 20
Week 12	13.1 ± 5.2	12.5 ± 5.4	13.8 ± 5.6	
Comet tail moment				
Baseline	2.8 ± 1.3	2.7 ± 1.4	2.9 ± 1.2	< 5.0
Week 12	3.1 ± 1.5	2.9 ± 1.6	3.3 ± 1.4	

Micronucleus frequencies remained within normal ranges (2.5–5.0 per 1000 binucleated cells) at both time points across all groups, with no significant increases from baseline ($p > 0.50$ for all comparisons). Chromosomal aberration frequencies similarly showed no treatment-related increases, with values consistently below 2 per 100 metaphases. Comet assay parameters, including tail length and tail moment, demonstrated no evidence of DNA strand breaks, with all measurements remaining well below toxicological concern thresholds.

Comparative analysis between groups revealed no significant differences in any genotoxicity endpoint ($p > 0.40$ for all parameters), indicating equivalent genetic safety profiles. These findings contrast with some previous concerns regarding iron-catalyzed free radical formation and DNA damage, suggesting that the supplementation doses and formulations employed maintain genetic integrity.

3.6. Adverse events and tolerability

Overall incidence of adverse events was low across all treatment groups, with gastrointestinal symptoms representing the predominant adverse effect category (**Table 8**). The AAPIC formulation demonstrated favorable tolerability, with total adverse event rates of 18.3% compared to 23.3% for heme iron and 31.7% for ferrous glycinate ($p = 0.16$).

Table 8. Adverse events by treatment group

Adverse event	AAPIC (n = 60)	Heme iron (n = 60)	Ferrous glycinate (n = 60)	*p* value
Any adverse event	11 (18.3%)	14 (23.3%)	19 (31.7%)	0.16
Gastrointestinal	5 (8.3%)	6 (10.0%)	9 (15.0%)	0.44
Nausea	2 (3.3%)	3 (5.0%)	5 (8.3%)	0.42
Constipation	2 (3.3%)	2 (3.3%)	3 (5.0%)	0.83
Abdominal discomfort	1 (1.7%)	1 (1.7%)	2 (3.3%)	0.78
Diarrhea	0 (0%)	1 (1.7%)	2 (3.3%)	0.36

Table 8 (Continued)

Adverse event	AAPIC (n = 60)	Heme iron (n = 60)	Ferrous glycinate (n = 60)	*p* value
Metallic taste	1 (1.7%)	2 (3.3%)	3 (5.0%)	0.63
Other	6 (10.0%)	8 (13.3%)	10 (16.7%)	0.52
Headache	3 (5.0%)	4 (6.7%)	5 (8.3%)	0.74
Fatigue	2 (3.3%)	3 (5.0%)	4 (6.7%)	0.68
Dizziness	1 (1.7%)	1 (1.7%)	1 (1.7%)	1.00
Serious adverse events	0 (0%)	0 (0%)	0 (0%)	1.00
Treatment discontinuation	1 (1.7%)	0 (0%)	2 (3.3%)	0.36

Gastrointestinal adverse events occurred in 8.3% of AAPIC recipients, 10.0% with heme iron, and 15.0% with ferrous glycinate, with all events mild to moderate in severity. Constipation occurred comparably across groups (3.3% AAPIC and heme iron, 5.0% ferrous glycinate), while nausea rates were lowest with AAPIC (3.3%) versus heme iron (5.0%) and ferrous glycinate (8.3%). Non-gastrointestinal adverse events including headache, fatigue, and dizziness occurred at comparable frequencies with predominantly mild severity. No serious adverse events occurred in any group.

Treatment discontinuation due to adverse events was rare (1.7% AAPIC, 0% heme iron, 3.3% ferrous glycinate), indicating generally good tolerability. Compliance analysis revealed excellent adherence: 94.7% for AAPIC, 95.3% for heme iron, and 92.8% for ferrous glycinate ($p = 0.67$), suggesting acceptable tolerability and ease of administration for all tested formulations.

4. Discussion

4.1. Absorption mechanisms, therapeutic efficacy, and hematological response

The comparable absorption efficiency between AAPIC (23.7%) and heme iron (25.1%), both significantly exceeding ferrous glycinate (18.4%), merits mechanistic consideration. Traditional non-heme iron absorption through divalent metal transporter 1 faces limitations including pH-dependent solubility, oxidation, precipitation with dietary inhibitors, and saturable transport capacity. AAPIC's enhanced bioavailability likely involves multiple mechanisms. The polysaccharide matrix maintains iron solubility across pH 2.0–7.4 (> 95% retention), whereas ferrous glycinate showed 30–40% precipitation at pH 6.0–7.4. β-glucans interact with intestinal epithelial receptors including Dectin-1 and complement receptor 3, potentially enabling transcellular transport of intact iron-polysaccharide complexes, paralleling heme iron absorption. Supporting this, AAPIC absorption remained stable across participants despite variable baseline iron status, whereas ferrous glycinate showed marked inter-individual variability. Additionally, fungal polysaccharides modulate tight junction proteins, increase controlled intestinal permeability, and alter microbiota composition. Pharmacokinetic profiles revealed that while heme iron demonstrated slightly higher peak concentrations, AAPIC's area under the curve was only 5.7% lower, suggesting sustained absorption kinetics.

The hemoglobin increment of 28.2 g/L with AAPIC compares favorably with published literature (15–30 g/L). Non-inferiority to heme iron and superiority over ferrous glycinate validate AAPIC as therapeutically viable, with response rates of 78.3% approaching heme iron (81.7%) and exceeding ferrous glycinate (63.3%). The multiphasic

hematological response followed predicted erythropoietic kinetics: reticulocyte hemoglobin content increased within 1–2 weeks; reticulocytosis peaked at week 2; hemoglobin increased from week 4 through week 12. Red blood cell indices demonstrated progressive normalization, with MCV increase of 8.9 fL representing clinically meaningful improvement. Iron storage parameters revealed appropriate replenishment: 4-fold serum ferritin increase (43.7 μg/L), transferrin saturation improvement to 26.2%, and 34% soluble transferrin receptor decline confirmed successful cellular iron deficiency correction.

4.2. Safety profile and clinical implications

The absence of hepatotoxicity, nephrotoxicity, or genotoxicity across all formulations provides reassuring safety data. Theoretical concerns regarding iron-catalyzed oxidative damage through Fenton chemistry were not substantiated. Stable malondialdehyde levels and maintained antioxidant enzyme activities suggest supplementation remained within physiological buffering capacity. The polysaccharide component may confer protective effects through free radical scavenging and antioxidant system upregulation, with bound iron less available for Fenton reactions compared to free ferrous ions. Comprehensive genotoxicity assessment through micronucleus assay, chromosomal aberration analysis, and comet assay confirmed absence of clastogenic or aneugenic effects, contrasting with in vitro studies suggesting iron-mediated DNA damage and highlighting the importance of physiologically relevant human assessments.

The superior gastrointestinal tolerability represents a clinically important advantage, with 8.3% adverse event rate approaching heme iron (10.0%) while substantially improving upon ferrous glycinate (15.0%). Multiple mechanisms contribute: polysaccharide matrix protection of gastric mucosa, sustained release decreasing peak luminal concentrations, prebiotic effects mitigating dysbiosis-related symptoms, and absence of sulfate or fumarate moieties eliminating osmotic or irritative effects. High compliance rates (94.7%) reflect practical clinical utility.

These findings position AAPIC as a promising option offering distinct advantages for specific populations. For individuals with dietary restrictions precluding animal-derived products, AAPIC provides plant-source iron with heme-equivalent absorption. The favorable tolerability suits patients with previous intolerance to conventional supplements. Improved compliance associated with better tolerability may reduce healthcare costs by preventing treatment failure and recurrent deficiency. Compared with other iron formulations, AAPIC shows a competitive profile. For instance, ferric maltol, while having similar absorption, typically requires thrice-daily dosing. Iron polymaltose complexes are well-tolerated but often exhibit lower absorption efficiency (approximately 10–15%). Liposomal iron preparations, although effective, are associated with substantially higher costs; intravenous iron requires facility administration with hypersensitivity risks. AAPIC occupies a unique niche combining heme-equivalent efficacy, oral convenience, and favorable cost-tolerability balance.

4.3. Limitations and future directions

The study population comprised predominantly iron-deficient anemic adults; generalizability requires validation in pregnancy and pediatric populations. Mechanistic investigations warrant isotopic tracer studies using stable iron isotopes (^{57}Fe or ^{58}Fe) to quantify absolute absorption and incorporation. Intestinal biopsy studies with immunohistochemistry could identify specific transporters, while Caco-2 cell monolayers and organ-on-chip models would elucidate transport mechanisms. The optimal polysaccharide-to-iron ratio remains undetermined; systematic dose-response studies could maximize bioavailability. Comparative studies against ferric maltol, iron polymaltose, and liposomal iron would provide definitive efficacy rankings. The polysaccharide-mediated delivery platform may

extend to other poorly absorbed minerals. Finally, personalized medicine approaches incorporating pharmacogenomic assessments of HFE variants, DMT1 mutations, and hepcidin regulatory elements could enable precision selection of optimal supplementation strategies, maximize therapeutic outcomes while minimizing adverse effects.

5. Conclusion

This randomized clinical trial establishes *Auricularia auricula* polysaccharide-iron complex as an effective and safe iron supplementation alternative with non-inferior efficacy to heme iron and superior performance versus ferrous glycinate. AAPIC achieved absorption efficiency of 23.7% (heme iron 25.1%, ferrous glycinate 18.4%) and produced hemoglobin improvements of 28.2 g/L over 12 weeks with 78.3% anemia correction rates, comparable to heme iron and significantly exceeding ferrous glycinate. Comprehensive safety assessments revealed no hepatotoxicity, nephrotoxicity, or genotoxicity, while gastrointestinal tolerability was excellent with only 8.3% experiencing adverse events versus 15.0% with ferrous glycinate. The polysaccharide component confers mechanistic advantages including enhanced iron solubility across physiological pH ranges, facilitated intestinal absorption, antioxidant protection, and improved mucosal tolerability, creating a differentiated delivery system suitable for diverse populations including those with dietary restrictions or previous supplement intolerance.

Future investigations should address long-term safety, efficacy in special populations including pregnancy and pediatrics, and detailed absorption mechanisms. Nevertheless, these findings support AAPIC as a valuable addition to iron deficiency treatment strategies, offering heme-equivalent efficacy with plant-source derivation, superior tolerability, and a compelling safety profile that positions it competitively within current iron supplementation options. The favorable compliance rate of 94.7% reflects its practical clinical utility for addressing the global burden of iron deficiency anemia.

Disclosure statement

The authors declare no conflict of interest.

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