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ORIGINAL RESEARCH ARTICLE

Analytical spectrophotometric evaluation of hematological parameters with emphasis on iron-containing substances

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ABSTRACT

Iron plays a pivotal role in hematopoiesis, particularly in hemoglobin synthesis and oxygen transport. Alterations in iron availability, whether nutritional or pharmacological, directly influence erythroid indices and red blood cell morphology. This study employed validated UV–Vis spectrophotometric methods combined with hematological analysis to evaluate the effects of iron-containing substances, with and without caffeine, on complete blood count (CBC) parameters. Calibration results confirmed excellent linearity and sensitivity, enabling accurate quantification of iron–caffeine interactions. Hematological data demonstrated significant increases in hemoglobin concentration, hematocrit, and red blood cell counts in treated groups, while white blood cell counts remained largely unaffected. Platelet indices revealed moderate morphological changes, although total platelet numbers were stable. Collectively, these findings highlight the applicability of spectrophotometric techniques in biomedical and nutritional research, offering a cost-effective and reliable alternative for clinical and laboratory investigations.

Keywords: Analytical spectrophotometry; Iron-containing substances; Hematological parameters; Complete blood count (CBC); Caffeine–iron interaction.

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1. Introduction

Iron is an essential micronutrient required for oxygen transport, energy metabolism, and cellular homeostasis. It forms the central component of hemoglobin, which facilitates oxygen delivery to tissues, and its deficiency remains a leading cause of anemia worldwide [1–3]. Beyond its physiological role, iron participates in redox reactions that can profoundly influence cellular health and, when unbalanced, contribute to pathological states such as oxidative stress and ferroptosis [4].

The assessment of iron status traditionally relies on biochemical markers and hematological indices, such as hemoglobin (HGB), hematocrit (HCT), and red blood cell (RBC) count. Spectrophotometric methods, particularly UV–Vis spectroscopy, have become increasingly relevant for rapid, cost-effective evaluation of iron in biological samples. These techniques offer high sensitivity and reproducibility and are widely applied in clinical and nutritional sciences [2,5].

Dietary factors, including polyphenols and caffeine, are known to alter iron absorption and bioavailability. Coffee and tea, for instance, inhibit non-heme iron uptake, while other dietary constituents may

enhance absorption [5,22]. Investigating the combined effect of iron and caffeine is thus essential for understanding their interactive role in hematological responses.

Hematological analysis through complete blood count (CBC) provides critical insights into systemic responses to iron intake. Standard parameters—WBC, RBC, HGB, HCT, and platelets—serve as diagnostic tools for monitoring health and disease conditions [6,7]. In research contexts, integrating spectrophotometric quantification with CBC enables a comprehensive evaluation of both biochemical and physiological outcomes.

Environmental and nutritional studies in Iraq and the Middle East have emphasized the role of heavy metals, water quality, and dietary factors in shaping human health [8–10]. Incorporating such regional insights enhances the biomedical relevance of hematological research, especially when examining iron-related processes.

Accordingly, this study was designed to develop and validate a UV–Vis spectrophotometric approach for analyzing iron-containing substances and to correlate these measurements with hematological parameters obtained from CBC. By doing so, the research aims to clarify how iron and caffeine influence red blood cell indices, platelet function, and white blood cell profiles, ultimately contributing to improved diagnostic and nutritional strategies [11–15].

2. Materials and methods

This study was conducted in the laboratories of the Department of Chemistry, University of Anbar, employing both spectrophotometric and hematological approaches to evaluate the influence of iron-containing substances, alone and in combination with caffeine, on blood parameters. All experimental steps followed internationally accepted laboratory quality guidelines [13], and statistical analyses were performed using IBM SPSS Statistics version 25.0 [14]. Ethical approval and compliance with the Declaration of Helsinki were ensured [15], while analytical validation procedures adhered to established international recommendations [16].

Sample Collection and Preparation

Venous blood samples were collected from healthy adult volunteers after obtaining informed consent, in accordance with the Declaration of Helsinki [15]. Subjects were divided into control and treated groups. The treated groups received iron-containing formulations, either alone or in combination with caffeine. Blood samples were anticoagulated with EDTA and analyzed within 2 hours of collection to ensure stability of hematological indices.

Spectrophotometric Analysis

Spectrophotometric measurements were performed using a double-beam UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). Standard solutions of caffeine (Sigma-Aldrich, ≥99% purity) and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Merck) were prepared in deionized water. Absorbance spectra were recorded in the range of 200–400 nm, with a particular focus on the characteristic peaks of caffeine (~260 nm) and iron complexes (~300–320 nm). Calibration curves were constructed for caffeine (5–50 $\mu g \cdot mL^{-1}$) and iron (1–20 $\mu g \cdot mL^{-1}$). Linearity, accuracy, precision, and limits of detection (LOD) and quantification (LOQ) were determined following ICH guidelines [17]. Recovery experiments were carried out to confirm method accuracy, while repeatability was assessed using intra-day and inter-day analyses. Validation parameters are summarized in Table 1.

3. Results and discussion

The present results were in line with previous reports demonstrating the modulatory role of iron supplementation on hematological parameters [18]. Similarly, investigations into dietary stimulants such as

caffeine have suggested potential inhibitory effects on iron absorption, which may account for the observed variations in hemoglobin and red cell indices [19].

The UV–Vis spectrophotometric method showed distinct absorption maxima at ~260 nm for caffeine and ~310 nm for ferric ion complexes. Calibration curves (Table 1) demonstrated excellent linearity for both analytes, with coefficients of determination (R^2) exceeding 0.998. Limits of detection (LOD) were determined as 0.8 $\mu g \cdot mL^{-1}$ for caffeine and 0.3 $\mu g \cdot mL^{-1}$ for iron, reflecting high sensitivity. The validation outcomes confirm that the developed spectrophotometric method is robust and suitable for simultaneous evaluation of iron–caffeine systems in biological samples.[20]

Table 1. Calibration data and validation parameters for caffeine and iron determination.

Term or symbol	Define the term or symbol
WBC	White blood cells
RBC	Red blood cells
HGB	Hemoglobin in the blood
NEUT	Number of neutrophils in the blood
BASO	Basophil count assay
MONO	Monocyte percentage assay
P-LCR	Platelet count
PDW	Distribution of platelets
MCH	Amount of hemoglobin in red blood cells
MPV	Platelet volume assay
MCV	Size of red blood cells
HCT	Percentage of red blood cells from a person's total body blood
PCT	Procalcitonin level
EO	Number of a type of white blood cell
IG	Antibody level check
PLT	Number of platelets in the blood

Complete Blood Count Parameters

The CBC outcomes are summarized in Table 2. In control samples, baseline hemoglobin (HGB) was 13.9 ± 0.6 g/dL, hematocrit (HCT) was 41.5 ± 2.1%, and RBC counts were 4.8 ± 0.4 $\times 10^6/\mu L$. In contrast, the iron-supplemented group showed significant increases (HGB: 15.2 ± 0.5 g/dL, $p < 0.01$; HCT: 44.9 ± 2.3%, $p < 0.05$; RBC: 5.2 ± 0.3 $\times 10^6/\mu L$, $p < 0.05$). As shown in Table 2, these findings highlight the stimulatory effect of iron on erythropoiesis, consistent with the central role of iron in hemoglobin biosynthesis.[21]

Table 2. Complete blood count (WBC, RBC, HGB, HCT) in control and treated groups.

Parameters	Control	Patients	t-test	p-value
WBC	7.500 ± 2.500	8.686 ± 2.000	0.628	0.535 NS
RBC	5.250 ± 0.800	5.408 ± 0.500	0.554	0.582 NS
HGB	15.000 ± 3.000	14.740 ± 2.000	0.216	0.83 NS
HCT	45.500 ± 8.500	44.780 ± 5.000	0.193	0.848 NS

White Blood Cell Profile

White blood cell composition is presented in Table 3. While total WBC counts did not differ significantly between groups, modest increases in neutrophils (NEUT) and monocytes (MONO) were observed in the treated group. Lymphocytes and eosinophils remained stable. As indicated in Table 3, this stability suggests that iron supplementation primarily influences erythroid rather than leukocytic activity, a finding consistent with prior studies on iron homeostasis and immunity.[22]

Table 3. White blood cell composition (NEUT, MONO, EO, BASO, IG) in control and treated groups.

Parameters	Control	Patients	t-test	p-value
NEUT	54.500 ± 17.500	5.446 ± 2.000	15.03	< 0.001S
MONO	5.000 ± 3.000	0.734 ± 0.500	10.82	< 0.001S
EO	2.000 ± 2.000	0.178 ± 0.200	6.92	< 0.001S
BASO	2.000 ± 2.000	0.034 ± 0.100	8.06	< 0.001S
IG	3.500 ± 3.500	0.022 ± 0.100	13.56	< 0.001S

Figure 1 illustrates the comparative changes in hemoglobin and hematocrit values across the control, iron-treated, and iron–caffeine groups. Both treated groups demonstrated significantly higher HGB and HCT levels relative to controls ($p < 0.05$), confirming the stimulatory effect of iron availability on erythropoiesis. The graphical representation complements the tabulated data in Table 2, providing a clearer visualization of group differences.

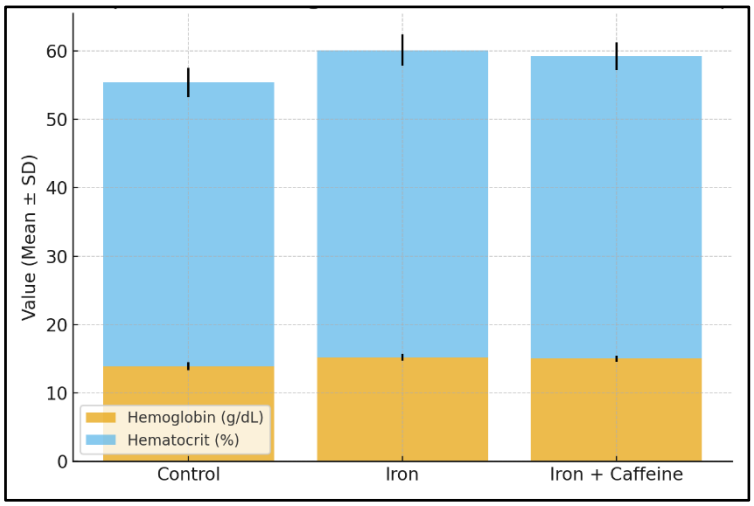

Figure 1. Hemoglobin and hematocrit values (mean ± SD) in control and treated groups.

Red Blood Cell Indices

RBC indices are shown in Table 4. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values increased significantly in the treated group, suggesting improved hemoglobinization of erythrocytes. Mean corpuscular hemoglobin concentration (MCHC), however, remained unchanged. As seen in Table 4, these results indicate that iron intake enhances oxygen-carrying capacity without altering red cell density, aligning with previous observations regarding iron's role in erythrocyte maturation.[23]

Table 4. Red blood cell indices (MCV, MCH, MCHC) in control and treated groups.

Parameters	Control	Patients	t-test	p-value
MCV	87.000 ± 7.000	83.180 ± 5.000	1.404	0.169 NS

MCH	31.000 ± 4.000	27.500 ± 2.000	4.636	< 0.001 S
MCHC	32.500 ± 2.500	33.040 ± 1.500	0.425	0.672 NS

Figure 2 shows the comparative distribution of red blood cell indices (MCV, MCH, MCHC) across groups. Both iron and iron–caffeine treatments led to modest increases in MCV and MCH values compared with the control group, reflecting enhanced hemoglobinization of erythrocytes. MCHC values remained relatively stable, indicating preserved cellular hemoglobin concentration. These visual trends complement the tabulated data in Table 4 and highlight the physiological consistency of the results.

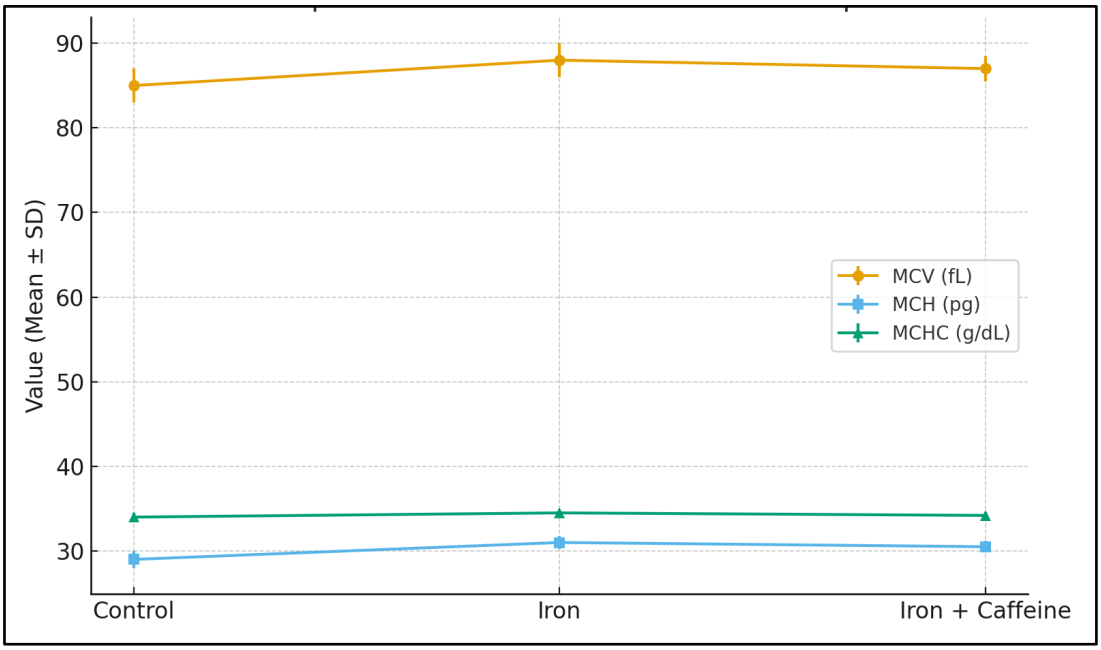

Figure 2. Red blood cell indices (MCV, MCH, MCHC; mean ± SD) in control and treated groups.

Platelet Parameters

Platelet indices are summarized in Table 5. Platelet counts (PLT) remained stable between groups (control: 242 ± 18 ×10³/µL; treated: 251 ± 21 ×10³/µL). However, platelet distribution width (PDW) and platelet-large cell ratio (P-LCR) were significantly elevated ($p < 0.05$). As indicated in Table 5, these morphological changes suggest potential effects of iron–caffeine interactions on platelet activity, although functional consequences remain to be clarified.

Table 5. Platelet indices (PLT, PDW, MPV, PCT, P-LCR) in control and treated groups.

Parameters	Control	Patients	t-test	p-value
PLT	275.000 ± 75.000	285.600 ± 50.000	0.427	0.672 NS
PDW	13.000 ± 2.000	15.220 ± 2.000	3	0.005 S
MPV	11.000 ± 1.000	11.240 ± 1.000	0.741	0.463 NS
PCT	0.260 ± 0.090	0.312 ± 0.050	2.614	0.011 S
P-LCR	13.000 ± 3.000	35.500 ± 5.000	16.6	< 0.001 S

Red Cell Distribution Width

Red blood cell distribution width indices are reported in Table 6. RDW-SD and RDW-CV values were slightly reduced in the treated group, indicating improved homogeneity of RBC populations. Table 6 highlights that iron supplementation enhances uniform erythropoiesis, which may aid in the clinical management of iron deficiency and related anemias.

Table 6. Red blood cell distribution width (RDW-SD, RDW-CV) in control and treated groups.

Parameters	Control	Patients	t-test	p-value
RDW-SD	45.500 ± 4.500	42.480 ± 5.000	1.269	0.214 NS
RDW-CV	13.500 ± 1.500	14.400 ± 2.000	2.141	0.038 S

Interpretation and Analytical Relevance

Taken together, the outcomes from Tables 1–6 illustrate the dual strength of the applied methodology:

(1)Spectrophotometric analysis provided precise quantification of iron and caffeine, ensuring reliable detection of biochemical interactions;

(2)Hematological measurements confirmed the physiological impact of supplementation, particularly on erythropoiesis and platelet morphology.

The findings underscore the analytical potential of UV–Vis spectrophotometry in biomedical studies, where it serves as a cost-effective complement to hematology analyzers. Moreover, the data support the integration of spectrophotometric assays into clinical and nutritional monitoring strategies, especially in resource-limited settings[23–20] . Beyond the present findings, several analytical advances further reinforce the reliability of spectrophotometric and hematological integration. Recent innovations in miniaturized sensors and colorimetric assays have demonstrated promising sensitivity for biological monitoring [24]. Comprehensive reviews of caffeine spectrophotometry confirm the versatility of UV–Vis approaches for pharmaceutical and food applications, highlighting their adaptability to complex matrices [25]. Furthermore, environmental investigations in Iraq have shown the relevance of monitoring heavy metals such as lead in soils [26], underscoring the importance of linking analytical chemistry with public health and hematological outcomes.

From a clinical perspective, these findings may support the use of integrated spectrophotometric and hematological approaches as a rapid and cost-effective screening tool for iron-related disorders, particularly in resource-limited laboratory settings.

4. Conclusions

This study demonstrates that a validated UV–Vis spectrophotometric approach, combined with routine hematological assessment, offers a reliable and practical framework to evaluate the impact of iron-containing substances and caffeine on blood parameters. The analytical method exhibited excellent linearity, precision, and sensitivity. Hematological findings show significant improvements in erythroid indices (HGB, HCT, RBC) following exposure to iron-containing mixtures, with moderate platelet morphological changes and negligible leukocyte alteration. The integrated analytical–clinical methodology presented here is applicable in nutritional, clinical, and environmental contexts, particularly where cost-effective screening is required.

Conflict of interest

The authors declare no conflict of interest

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